ATTACHMENT 3: Letter from B. Dementi - November 10, 1997

Clark Swentzel, Chairman November 10, 1997
HazardID SARC
Health Effects Division

As a follow-up to the November 6, 1997 HazardID SARC on malathion, I am compelled to express in writing my disagreement with certain very important decisions rendered at that meeting. One such issue concerns the apparent decision of the Committee to shift the basis of the RfD for malathion from the NOEL in the human study (Moeller and Rider, 1962), which has served in this capacity for years, to the NOEL for cholinesterase inhibition in the 1996 F344 rat chronic toxicity/carcinogenicity study. The problems I have with this decision are developed as follows. Firstly, the decision was too precipitous. By this I mean that since this is such a critical end point for this pesticide, it should have been presented as an issue or topic well before the meeting to allow people to be better prepared for discussion. I view this as a problem inherent in the process in dealing with a chemical having an extensive scientific record. Accordingly, there must be opportunity for offering further arguments supportable by additional information.

To the extent that Moeller and Rider incorporates a valid assessment of the LOEL/NOEL for cholinesterase inhibition in human subjects, being based as it is on **both** plasma and erythrocyte cholinesterases, evidence suggests humans are at least 10-fold more sensitive than F344 rats for erythrocyte cholinesterase inhibition and even more sensitive with respect to the plasma enzyme. To explain this difference, someone at the meeting suggested that 1962 vintage malathion was of questionable purity and that impurities could explain the differences with respect to the 1996 product. However, it was not indicated that humans have historically been more sensitive, i.e. were more sensitive than rat as compared on the basis of earlier products and likely remain so as compared to the more recent Cheminova product. Critical to the sensitivity of organisms to malathion in the cholinergic sense is the presence and level in such organisms of carboxylesterase activity, an enzyme(s) which, via catalysis of hydrolysis of one carboxyethyl group on malathion (actually malaoxon as the cholinesterase inhibiting entity), compromises its cholinesterase inhibitory capabilities. As I indicated at the meeting, insects lack carboxylesterase activity, which is thought to explain the remarkable selective efficacy of malathion as an insecticide. Similarly, to the extent that mammals incorporate differential levels of carboxylesterase activity they are variably sensitive to the agent in the cholinergic sense. Published works show that while carboxylesterase activity is located in the plasma and liver of the rat, in humans the enzyme is found in liver but not plasma. (Exhibit 1) The greater sensitivity of humans as demonstrated in Moeller and Rider may have its explanation in differing carboxylesterase activity in man versus rat. However, whatever the explanation, the fact remains that Moeller and Rider demonstrates the greater sensitivity of humans as compared historically using malathion of existing purity at the time and would likely prove so today if compared using the recent Cheminova product. I present these views as a way of dismissing any notions that Moeller and Rider has any fundamental flaw, if it can be accepted that malathion used in that study was at least as pure as 1962 vintage technical malathion, though purity of malathion used in the study was not provided. If it were a more highly purified product, then to the extent that such culprit cholinesterase inhibiting impurities as malaoxon and isomalathion were reduced, the concern about relative human sensitivity would be to that extent more enhanced.

In view of these considerations, greater scrutiny of the rat cholinesterase data than was had at the November 6 meeting would be essential before a shift could be made from human to rat data as the basis for deriving an RfD. Along these lines I have the following to say. The Cheminova malathion technical product is said to be more pure than the former American Cyanamid product. Before the Committee accepts such claim, members should have in hand the Confidential Statement of Formulation for the respective products for direct comparison by the Committee. This is particularly important with respect to levels of cholinesterase inhibiting impurities. Cheminova has submitted data showing higher LD50 values for their product versus the American Cyanamide product, but LD50 may not be a good reflection of how products may compare at low levels of exposure based on cholinesterase data. LD50 values may be confounded by a host of adverse effects of the test material including cholinesterase inhibition brought on by trace impurities of cholinesterase inhibiting entities that do not require activation and thus become relatively more important at high doses of malathion where metabolic conversion of malathion to malaoxon becomes more saturated. Actually, I must confess to the committee that I very carefully compared the two product compositions awhile ago and there are reduced levels of malaoxon and isomalathion in the Cheminova product versus the American Cyanamid product, but I would question the relative effects of these these entities at low doses where metabolic conversion of malathion to malaoxon is less saturated.

In developing the protocol for the recently (1996) submitted malathion chronic/carcinogenicity study, the registrant was advised by our staff that 100 ppm, which the registrant was proposing as a low dose for the study, included principally in search of a NOEL for cholinesterase inhibition, would likely not be a NOEL for the blood borne cholinesterases. (Exhibit 2) It was explained that 100 ppm (lowest dose tested) was not a NOEL in the 1980 chronic/carcinogenicity study in the Sprague-Dawley rat, and likely would not be a NOEL in the new study. Nontheless, the registrant elected 100 ppm as the low dose for the new study, partly predicated on their view that their product is more pure than the American Cyanamide product empolyed in the earlier studies. As it developed, after 3 months on test, statistically significant erythrocyte cholinesterase inhibition was observed in females, prompting a reduction of the low dose to 50 ppm for rats of both sexes for the duration of the two year study in search of a NOEL. (Exhibit 3) I should note at this point that this finding corroborated the finding in the Sprague-Dawley rat performed seventeen years ago using tha American Cyanamid product. Subsequent to the three month time point, 50 ppm proved to be a NOEL for erythrocyte cholinesterase for both sexes. Firstly, what this says to me is that there is little if any improvement in the Cheminova product over that of the American Cyanamide product with respect to inhibition of erythrocyte cholinestyerase at low doses, particularly those critical to setting the RfD for malathion. Secondly, in the DER for the new chronic/carcinogenicity study in the rat, additional cholinesterase information is called for in view of the absence of a NOEL for cholinesterase inhibition among females at the 3 month time point. It is alleged in the DER that given the ability of organisms to adapt somewhat to cholinesterase inhibitors (see, for example, the recovery of erythrocyte cholinesterase inhibition for females at 500 ppm at 6 months in that study, Exhibit 4), there is no assurance that the enzyme would not have been inhibited at 50 ppm during the first three months, i.e. during a very critical time frame for exposure to a pesticide. This is also very important in view of the facts that, a) malathion has a very shallow dose response curve (in my judgement there is very little difference between 50 and 100 ppm for an agent that demonstrates such a shallow dose response curve ranging up to 6000-12000 ppm), b) the human study demonstrated greater sensitivity for uncertain reasons and c) the number of animals assayed for cholinesterase activity, 10/sex, does not accord sufficient statistical power to clearly identify a NOEL at low but meaningful levels of inhibition. I must maintain at this point that a definitive NOEL for cholinesterase inhibition be determined over at least a three month period using large numbers of rats at doses that embrace those employed in Moeller and Rider (.11-.34 mg/kg/day) overlapping those of the lower dose range of the rat chronic/carcinogenicity study, say up to 20 mg/kg/day. To the extent that this end point will be employed in establishing the RfD for malathion, I view it imperative that this data be gathered.

In summary I consider it inappropriate to change the basis of the RfD for malathion from the Moeller and Rider human study to the recently submitted chronic toxicity/carcinogenicity study in the F344 rat, particularly without a definitive NOEL for cholinesterase inhibition over the first three months of testing in ths case of the rat. Also, I recommend additional study to obtain a more definitive NOEL for cholinestarase inhibition at low doses in the rat

cc Jess Rowland

Brian Dementi
Toxicologist, HED.

ATTACHMENT 4: Letter from B. Dementi - November 20, 1997

Clark Swentzel, Chairman November 20, 1997
Hazard ID SARC
Health Effects Division

Re: Ad Hoc Committee Meeting of November 13, 1997 on Malathion Issues

As a matter of the record, regarding the referenced meeting, this is to advise you that in spite of the good effort on your part to see that a fair and reasonable meeting was held, and I thought you did well, I do not consider the outcome satisfactory. The decisions made were very inadequate and not in the interest of the public health, as they compromise full pursuit of the understanding of the toxicology profile on this important and extensively used pesticide. No stone should be left unturned, given the enormity of human exposure to this cholinesterase inhibiting organophosphate. I shall comment on the topics that were the subject of the meeting in the order in which they were taken.

Retinal Anomaly in Acute Neurotoxicity Study on Malathion (MRID 43146701

I have presented fully my views on this subject in written documents, which were available to the Committee, and will not restate these views at this writing. The fact remains that the Acute Neurotoxicity Guidelines (81-8) call for sequential histopathologic evaluations of specific tissues in lower dose groups when histopathologic findings are noted in the high dose group animals. It would appear to me that this requirement should be met in this Guideline even if but one lesion is observed in a particular tissue of the high dose group given the small number of animals (5/sex) in a dose group. This was not done in the study in question after the one bilateral retinal rosette was noted in a high dose male group. Now it is not a source of happiness to me to be perceived as one who over-assesses a study, and this is why I feel very awkward in defending this position. If the one incident standing alone had been identified among fifty or more animals in a group, surely I would not have pursued the matter, but in this case given the rarity of the lesion in historical data bases and the uncertainty as to the lesions microscopic anatomic features (retinal rosette is not an anatomic term and on the face of it, the term could be used to apply to any of a variety of underlying morphologic changes), I felt that as a matter of the record, our pathologist should provide anatomic characterization. Also, there was somewhat greater incumbency to require this assessment since it involved the retina, in view of the prevailing concerns over possible retinal effects of organophosphates in general and of malathion in particular. While I did not say so at the November 13 meeting, it is essentially self-evident that the assesment of the requested slides could be instrumental in determining whether to insist upon examinations of lower dose groups as mandated in the Guidelines. For example, this might be contingent upon whether the bilateral retinal rosette of the high dose male in the acute study is morphologically or anatomically the same as that of the unilateral rosette of a control rat in the subchronic neurotoxicity study.

Lastly, I believe the relatively minor decision to ask for a couple slides should be entirely within the perview of the reviewer, given what may be his peculiar perspectives on the subject, without having it go before a committee for approval. As I said, for the record, this issue remains unresolved if the slides in question are not submitted.

Relative Sensitivity of Females Versus Males to Cholinesterase Inhibition by Malathion

I presented to the Committee several comparisons of the level of cholinesterase inhibition for males and females from our Guideline and dose range-finding studies on malathion and malaoxon. Although the magnitude of differences between the sexes is variable across studies, there is more than adequate evidence to establish a greater sensitivity for females. The ad hoc Committee did agree that sex-related differences are manifest, but did not concur with the proposition that differences may merit a correction factor to be applied to male (human) data used as the basis for the RfD. It should be noted at this point that the RfD for malathion, 0.02 mg/kg/day, which ostensibly protects the entire human population - men, women, boys and girls of all ages- employs a mere ten-fold safety factor as applied to experimental data obtained on humans (men only). In the absence of such data for women and youths, in my judgement a larger safety factor than ten should be employed, particularly in the face of evidence that females are more sensitive to malathion than males as assessed in laboratory animal studies, and where studies of organophosphates in general suggest young individuals to be more sensitive. According to the 1988 malathion registration standard: "The Theoretical Maximum Residue Contribution (TMRC) for the U.S. population average is 0.1014 mg/kg/day, occupying 505% of the PADI. For children 1 to 6 years of age, the TMRC occupies 1133% of the PADI. The TMRC is based upon current tolerance levels and an assumption that 100% of the sites are treated. Actual dietary exposure may be much lower." (p.32) The point is that a much higher percentage of the PADI is consumed, or was so in 1988, than is to be desired, which places an enhanced scrutiny upon the reliability of the RfD in protecting real people.

Unfortunately I did not have the time before the meeting to provide study by study estimates of such correction factors, but am certain that a legitimate correction factor, whatever it is, would be of such magnitude that it should not be ignored, especially in view of the small safety factor used for the existing RfD. Additional study in animals may be necessary to properly identify the correction factor. Realizing that a sex-related differential sensitivity exists, unacceptable in my opinion is the Committee's out of hand rejection of the argument that a meaningful ratio exists without first obtaining some numerical estimates of that ratio of sensitivity from the data currently in hand. Indeed, I had anticipitated that an outcome of the meeting would be a Committee recommendation that such estimates be computed for subsequent consideration.

Testing for Effects on Learning/Memory

Again, available to the Committee were various documents presenting arguments pro and con that findings with malathion on learning/memory at very low doses in a published work, Desi et al. (1976), are of sufficient validity and concern to require Guideline testing of malathion for these effects. In addition to explaining to the Committee that the published work shows that malathion at doses of 38-75 mg/kg/day in a subchronic study elicited effects on learning/memory, EEG and EMG, as contrasted with no neurotoxic (motor activity, FOB parameters) effects in the Guideline subchronic neurotoxicity study at doses up to 1575 mg/kg/day, I had recommended that a Guideline test of learning/memory be required for malathion. The Committee rejected this recommendation on the grounds that Desi et al (1976) is not a reliable study. This criticism of the study was maintained in spite of many findings in the study that affirm its veracity. Of these I mentioned the facts that the stated purpose of the authors was to assess the effects of malathion at subclinical levels on sensitive neurotoxicity parameters including learning/memory; 95% malathion (American Cyanamid) was used; the authors affirmed the absence of clinical signs which was consistent with the low but meaningful level of cholinesterase inhibition; cholinesterase activity was remarkably well evaluated in the study, including assessments of plasma, erythrocytes and brain regions, where the findings were consistent with those of the Guideline subchronic neurotoxicity study (which in turn enhances the credibility of the published work), and adverse effects of malathion on kidney tissue in in vitro kidney tissue cultures being somewhat consistent with or supported by chronic nephropathy as the cause of increased mortality (100% and 74% in the high and penultimate doses,

respectively) in the 1996 chronic toxicity/carcinogenicity study in the F344 rat. Furthermore, the authors of the study affirm in the text a real effect of malathion on learning and memory as assessed in their study.

The Committee members were mute with respect to acknowledging any of these facts as supporting evidence of the work by Desi et al, but persisted in criticizing the study on the grounds that the effects on learning/memory in terms of errors made by rats in maze studies were small, not dose related between 38 and 75 mg/kg/day; that statistics were ill defined and that it would be surprising for malathion to exert such an effect at such low dose levels. I endeavored to explain that findings were in fact not small in terms of differences in errors made in dosed groups versus controls. I also offered my opinion that 38 and 75 mg/kg/day, when compared on the shallow dose response for malathion are actually not very different, and that brain cholinesterase inhibition was 20% in the two groups at 21 days, the time at which learning/memory was affected. These two observations would point to similar responses on tests of learning/memory, and thus the absence of a dose response as noted. I also explained from an earlier work by Desi et al, which the authors cited as background for methodology, that bar graphs in that study were said to be standard deviations, which if true in the 1976 study would mean that differences between controls and dosed groups on errors made in the learning /memory test would be statistically significant. In spite of these findings, plus the EEG and EMG data affirming a neurological effect of the test material at these dose levels, and in view of the fact that the Guideline subchronic neurotoxicity study was not designed to assess learning/memory, EEG or EMG effects that could refute the findings in Desi et al, the Committee categorically rejected the Desi study as of any relevance. In fact, I recall saying to the group, "It's as if Desi does not exist?", whereupon I was responded to in the affirmative. In my judgement, this qualifies as an authoritarian rejection of data the Committee failed to refute. I maintain that Desi et al (1976) in spite of its deficiencies is of sufficient quality that it conclusions, particularly with respect to the effects of malathion on learning/memory, mandate verification through proper Guideline testing procedures, which are available. As to the question of the "small" effect on errors made by rats in the learning and memory aspect of Desi, et al, one might ask, what is small? Imagine a high school student taking his algebra exam, on which his grade would be say 97, other things being equal, but under the influence of a xenobiotic he was exposed to, his score turned out to be 92 due to a few additional errors he made. Now a 92 (B) is a very good grade, but not quite as good as the grade he deserved 97 (A). One might say this is a small difference, but who would argue that is to be ignored?

I have concerns about the legitimacy of the opportunity presented to me to go before an unbiased ad hoc committee. I had reservations before the November 13 meeting that I should even pursue the matter. This concern was born out by the following episode that occurred at the meeting. As you will recall during the meeting, at the precise moment that we completed our deliberations on the second topic, one Committee member, arriving late, voted on the issue. In fact, as I recall, you commented at the time that so and so is voting even though she was not present during the discussion. From my perspective, her vote was more than improper in that it conveyed the impression, whether rightly or wrongly interpreted, that the Committee's conclusions were foreordained, and that my opportunity to be heard at this meeting was a mere formality. When I came to item three, my presentation was compromised in the psychological or motivational sense, given what had previously taken place. I could see "The handwriting on the wall" and thus the futility in proceeding further on what was really the most important of the three issues. In my view, minds had been made up, and I felt nothing I said would matter before this Committee. Indeed, I came preciously close to calling off any further discussion, but felt that would be of no avail either, as people might then say "well, you had your chance", as if this were some kind of real and legitimate peer review. I am convinced it was so in name only. The bottom line to all this is that another forum for peer review of these issues is required, bearing in mind the importance of this subject to the public health. People composing a true peer review committee should be experts in the field, but at the same time should not have personal vested interest in HED.

Brian Dementi
Toxicologist, HED

cc Jess Rowland

ATTACHMENT 5: Letter from B. Dementi - November 25, 1997

Clark Swentzel, Chairman
Hazard ID Committee

November 25, 1997

RE: Malathion RfD

It is my intent here to comment further on certain issues before the Hazard ID SARC of November 6 and the Ad Hoc Committee meeting of November 13, 1997, with particular reference to the RfD for malathion.

In my memorandum to you of November 10, I endeavored to explain why the cholinesterase data in the recent chronic toxicity/carcinogenicity study of malathion is inadequate to define a NOEL for female F344 rats. As a remedy, I recommended a definitive three month assessment of cholinesterase inhibition in the rat. In my judgement, until such data are available, a gap exists with respect to the identification of a NOEL for the first three months of exposure to malathion, and, hence, proper data do not exist in this study upon which to poise an RfD. This being true, and to the extent that the Moeller and Rider (1962) study, performed in humans, may continue to be used as the basis for the RfD until proper rat data are obtained, the following comments are relevant.

At the Ad Hoc Committee meeting, when discussing the topic of greater sensitivity of females to cholinesterase inhibition by malathion, I expressed the view that for studies wherein cholinesterase inhibition was obtained in but one sex, as is true in Moeller and Rider where only male volunteers were tested, that a greater than the normal uncertainty factor (UF) of 10 should be applied. As I recall, this was not affirmed by any one at the meeting. I suspect no one felt sufficiently certain to render a definite opinion. In any case, I believe this is a question requiring an answer. I do not have the time to search the records, but I believe the answer should be readily available in the minutes of past RfD meetings, and should be a well recognized operating principle for the RfD Committee. I have just by chance reviewed the 1997 Registration Eligebility Document (RED) toxicology chapter for carbofuran, and I find in the case of the RfD that the Agency applied a UF of 100 to the NOEL for cholinesterase inhibition in male volunteers. Quoting from that RED chapter: "An uncertainty factor (UF) of 10 was applied to account for intra-species variability. An additional UF of 10 was applied to account for study deficiencies (use of limited number of subjects, few subjects/dose and **use of males only** (emphasis added)". Please be aware that Moeller and Rider, in addition to being a study in males only, has its inadequacies also (e.g., limited number of subjects, purity of the test material not provided, interpretation of low and mid dose effects somewhat confounded by co-administration of EPN).

In my memorandum to you of November 20, I quoted from the malathion registration standard, passages revealing how high the TMRC is (or was in 1988) when based on the RfD of 0.02 mg/kg/day, derived from Moeller and Rider with a UF of only 10. The Committee should be aware that at an earlier time point, a UF of 100 had been applied to Moeller and Rider, at which time the RfD was thus 0.002 mg/kg/day. Also at that time the TMRC was about 5000% of the PADI. At some point in time, and I don't have the details, I would estimate around 1987-90, the UF was reduced from 100 to 10, for reasons unknown to me. I recommend that your Committee seek the historical record on the setting of the RfD for malathion, and make your own independent assessment of its reasonableness, as this is the moment in time for reconciling the RfD with the facts at hand. On the face of it, if a UF of 100 is appropriate for carbofuran for the reasons given, an explanation should be forth coming for the use of only 10 in the case of

malathion. Please understand I am not saying a satisfactory explanation does not exist, but let us see it. I must maintain the view that when a UF of only 10 is employed, it is imperative that the study in question incorporate data on both sexes.

In summary, in my view proper data on cholinesterase inhibition in rats are not available at this moment to justify replacing the Moeller and Rider human study as the basis for the RfD for malathion. Furthermore, in the absence of cholinesterase data on women, the UF as applied to the Moeller and Rider human (men only) data should be revised upward from the 10 which is currently employed.

Brian Dementi, Ph.D.
Toxicologist/HED

cc Jess Rowland
George Ghali

ATTACHMENT 6: Letter from B. Dementi - December 17, 1997

Jess Rowland, Secretary
Hazard ID Committee

December 17, 1997

Comments on December 4, 1997 draft report of malathion Hazard ID Committee meeting of November 6, 1997. The following is the best I am able to produce given the constraints of time and the complexity of the subject.

Comments on the various endpoints are presented as follows in the order in which they appear in the draft report.

I Introduction (p. 1) O.K.

11 Hazard Identification

A. Acute Oral (one-day): For this endpoint, the Committee concluded that the 50 mg/kg/day dose is appropriate for acute dietary risk assessment. This endpoint is based upon decreased maternal body weight gain in the malathion developmental toxicity study in the rabbit (MRID 152569). In support of this, the draft Hazid ID Committee Report (HIDR) cites the DER for the rabbit developmental toxicity study as showing a LOEL/NOEL of 50/25 mg/kg/day. However, it must be recognized that the DER concluded this conditionally upon receipt of Appendix III (DER p. 7), which contains individual animal data and was not included with the study MRID. This Appendix was submitted later as part of MRID 40812001, which includes the full study as well. I am not certain whether this individual data was evaluated by anyone in HED. It was explained in the Der (p. 6) that the non-statistically significant maternal body weight gain decrease at the low dose (25 mg/kg/day) could not be adequately evaluated due to the absence of individual animal data located in the missing Appendix III. As cited in the HIDR (p. 3), mean body weight gain during days 6-18 of gestation were 0.19, 0.06, - 0.03 and - 0.03 kg at 0, 25, 50 and 100 mg/kg/day, respectively. In order to evaluate statistically the numerical decrease at the low dose level vs. Control, i.e. 0.06 vs 0.19 kg, the individual data would be needed. Furthermore, the DER claims that the decrease seen at the low dose was principally accounted for during days 6-12 and that during days 12-18 the low dose dams actually gained more weight than controls. According to the study report, body weight gain during gestation days 6-12 were 0.08, -0.04, -0.02 and -0.06 kg for control, 25, 50 and 100 mg/kg groups, respectively, where none of the dosed groups were reported as statistically significant with respect to control. (MRID table 3, p. 18).

In my opinion the data should be more closely examined before concluding where the LOEL/NOEL lies in this study, particularly if this end point is to serve as the basis for acute dietary risk assessment.

The HIDR says that there were no decreases in body weight gain at 50 mg/kg/day in the Range-Finding study. (P. 5). However, inspection of doe body weight gain data in the range-finding study shows body weight was not significantly altered at any dose level up to and including the highest dose of 400 mg/kg. (MRID 152569, table 3, p.16). Evidently, the reasons for this lack of a finding of an effect on body weight gain include the small number of animals employed and the high variability in body weight data. I do not see how this data can be cited in support of any conclusion with respect to effects of the test material on doe body weight. Furthermore, before concluding that a single dose as high as 50 mg/kg would not elicit a meaningful biological effect one should have cholinesterase data over several days following that single dose. In a journal publication mentioned in DER #11, p. 11 provided the Committee, it is noteworthy that as assessed in the Sprague-Dawley rat where malathion (American Cyanamid 95% t.a.i) were administered intraperitoneally at single doses of 0, 25, 50, 100 or 150 mg/kg, avoidance behavior was significantly impaired 1 hour after injection with 50 mg/kg and above. There were no clinical signs observed over a

24-hour post-dosing period at any dose excepting one rat in ten at the 150 mg/kg group, which exhibited tremors. Cholinesterase inhibition was significantly inhibited only at 100 and 150 mg/kg during the 24-hour period, so the author concluded that low doses of malathion may disrupt behavior without significantly reducing cholinesterase activity [Kurtz, P. J. (1977) Dissociated Behavioral and Cholinesterase Decrements following Malathion Exposure, Toxicol. Appl. Pharmacol. 42, 589-594]. The behavioral effect found in this study was remarkable as observed at the 1 hour post-dosing time point, but was not observed at 4 or 24 hour time points.

I do not accept that a developmental toxicity study provides sufficiently rigorous toxicologic data to serve as the basis for defining this critical end point. The absence of cholinesterase assessments in particular in these studies should preclude their use as the primary source of information for an end point as important as that for use in acute dietary risk assessment.

Acute Dietary Risk Assessment

The HIDR claims that the 10X factor to account for increased sensitivity of infants and children required under FQPA should be removed. This is rationalized on the grounds there is no evidence in the reproduction and developmental toxicity studies of increased sensitivity of developing and young animals. In the rabbit developmental toxicity study doses administered during gestational days 6-18 were 0, 25, 50 and 100 mg/kg/day. Similarly in the rat developmental toxicity study (MRID 41160901) doses administered during gestational days 6-15 were 0, 200, 400 and 800 mg/kg/day. We concur that in neither of these studies was there any evidence of increased sensitivity of the developing organisms with respect to the dams, insofar as the parameters evaluated were concerned. There is a serious question, however, whether such parameters are adequate to detect critical end points. The lowest dose used in both of these studies are well above those that inhibit cholinesterase in adult rats and rabbits. In the absence of cholinesterase assessments or clinical signs in the developing organisms versus those of the maternal animals, it is simply not possible to affirm that the developing organisms were not more adversely affected than the maternal animal. I am of the opinion that cholinesterase inhibition could have been more remarkably inhibited in selected developing tissue of fetuses, and furthermore, a given level of inhibition may be more deleterious in various ways in developing organisms that would not be found in the limited set of end points evaluated in developmental toxicity studies. On the face of it, though the developmental toxicity study is useful in detecting possible developmental anomalies, its capability is not sufficient to address possible cholinergic effects or cholinesterase inhibition, as these very fundamentally important parameters are simply not evaluated.

In the case of the reproduction study (MRID 41583401) concentrations administered via the diet for two generations were 0, 550, 1700,5000 and 75000 ppm. The low dose concentration in this study translates to 43 mg/kg/day for males and 51 mg/kg/day for females. The HIDR states that pups were no more sensitive than adults on the basis of such parameters as body weight, mortally, clinical signs. It is my observation that doses of 43-51 mg/kg/day and above would have resulted in cholinesterase inhibition, given the facts that the enzyme has been shown in other subchronic studies or time intervals to be inhibited at much lower doses, in fact. It is not particularly surprising that clinical signs were not observed except at the highest dose. In terms of clinical signs, rats tolerate cholineserase inhibition borne of malathion exposure remarkably well. As in the case of the developmental toxicity studies, the question is whether a differential inhibition between pups/young animals and adults would have been observed, and whether young individuals are more or less sensitive in terms of behavioral effects (a term that embraces many types of end points). These parameters are not evaluated in these types of studies. So I must reiterate the opinion that developmental and reproduction studies while perhaps adequate to assess the effects of chemicals on the parameters of primary interest in those studies, namely developmental and reproductive effects, such studies are not of the character needed to differentiate relative sensitivity of young and mature animals to satisfy FQPA concerns. The

absence of cholinesterase assessments is a most fundamental road block for this use of these studies. The elimination of the 10X factor cannot be justified except on crude and therefore risky terms from the public health perspective. There is evidence from various studies that young and developing animals have an enhanced sensitivity to cholinesterase inhibitors in general, attributable to cholinesterase inhibition [Pope, C. N. and Chakraborti, T. K. (1992) Dose-Related inhibition of brain and plasma cholinsterase in neonatal and adult rats following sublethal organophosphate exposures. Toxicol. 73, 35-43]. Therefore, there is incumbency to demonstrate that young animals are not more sensitive than adults to the effects of malathion on that very basis, namely, cholinesterase inhibition and behavioral consequences, which were not assayed in the very studies cited to rule out the possibility of greater sensitivity of young individuals.

It is a curiosity that in HIDR pp. 13-14 under the topic of Determination of Sensitivity, mention is made of the fact that cholinesterase data were not obtained for maternal animals nor their offspring or fetuses in the reproduction and developmental toxicity studies, without any attendant discussion of the implications of this lack of data. I believe the implications are precisely those expressed above, which is that without such data it cannot be said that young animals are no less sensitive than adults to the effects of malathion, and, hence, the elimination of the FQPA required 10X factor would be without justification.

B. Chronic Dietary [Reference Dose (RfD)]: This portion of HIDR shows the calculation of an RfD based upon plasma cholinesterase inhibition in the recent F344 rat chronic toxicity/carcinogenicity study (MRID 43942901). The problem I have with this is that it does not address the failure of that study to identify a NOEL for erythrocyte cholinesterase inhibition among females during the first three months of testing. My arguments are discussed in my November 10, 1997 memorandum to Clark Swentzel, Chairman of this Committee. I will not take the time to reiterate those views here, except to emphasize the importance of obtaining a definitive NOEL for cholinesterase inhibition as explained in the memo cited. Given the facts that erythrocyte cholinesterase was inhibited in female rats at 100 ppm and 500 ppm at the three month time point, but not at the 50 ppm or 500 ppm levels at the six month time point is inexplicable. Possible explanations are that there is adaptive recovery post three months (in which case 50 ppm is not a definitive NOEL for that initial three month period, a critical time frame) and too few animals were employed to obtain good cholinesterase data in view of the shallow dose response for malathion. Such possible explanations support conducting a definitive cholinesterase assessment over a three month time point using adequate numbers of rats to provide statistical resolution. Another <u>possible</u> explanation is flawed cholinesterase methodology, which if true may be a more fundamental problem not peculiar to malathion. The point is that until a NOEL for cholinesterase inhibition among females has been determined via a definitive study, the transfer of the RfD from the Moeller and Rider study in my opinion lacks adequate support.

The HIDR (p. 6) claims that the NOEL of the 2-year study is supported by the 90-day study. If this is in reference to the subchronic neurotoxicity study (MRID 43269501), it is true a NOEL of 50 ppm was found over the 90-day period, but that study employed but -5 rats/sex/group at each time point and had no other dose group between 50ppm and 5000 ppm that would demonstrate the ability of the study to detect cholinesterase inhibition within that large range. Furthermore, plasma cholinesterase inhibition is so imprecise in that study that it is questionable whether 5000 ppm or even 50 ppm is a NOEL in either sex, which underscore the need for a study on a large number of animals to obtain a definitive NOEL for cholinesterase inhibition.

In the mouse carcinogenicity study (MRID 43407201) there is no <u>NOEL</u> for liver histopathology in male mice, where the LOEL is 100 ppm (17.4 mg/kg/day). This study awaits a Pathology Work Group evaluation.

Chronic Dietary Risk Assessment : HIDR (p. 6) says that the Committee determined that the 10X factor should be removed. The reasons cited are the same as those for dropping the 10X factor from the acute risk assessment, namely the reproduction and developmental toxicity studies do not show a greater sensitivity of offspring or fetuses. To this I respond with the same arguments presented above in the case of the acute risk assessment, which is that it is not justified.

C. Occupational/Residential Exposure

1. **Dermal Absorption:** O.K.

2. **Short-Term Dermal - (1-7 days)** : O.K.

3. **Intermediate-Term Dermal (7 Days to Several Months)** : O.K.

4. **Long-Term Dermal (Seven Months to Life-Time) :** O.K.

5. **Inhalation Exposure (Any-Time Period):** The executive summary provided for the subchronic inhalation study is correct. I should emphasize that hyperplasia of the olfactory epithelium was described as locally extensive and that the olfactory/respiratory epithelial junction was severely affected in most animals. This means at all doses and there was no NOEL. The HIDR claims that since this study is the only inhalation study available in the toxicology data base, the LOEL will be used for short - intermediate - and chronic inhalation risk assessment. I view this as quite a burden for a study without a NOEL for both cholinesterase inhibition and nasal hyperplasia, but I have the greater concern for the hyperplasia aspect. **It is my opinion that this Committee should mandate a new inhalation study designed to identify a NOEL for histopathology of nasal tissues.** I say this not only because there was no NOEL, but because the hyperplasia is described as severe. There is a rational basis for a remarkable effect of malathion in particular on the olfactory epithelium, which is discussed at length in the DER for the recent malathion F344 chronic toxicity/carcinogenicity study (MRID 43942901). Briefly, the sensitivity of the olfactory epithelium to malathion rests with the remarkable metabolic capability of this tissue, as well as the unique structure of malathion as a diester of a dicarboxylic acid which may be hydrolyzed in the olfactory epithelium to yield carboxylic acids. The metabolic capability of the olfactory epithelium has been hypothesized as critical to the maintenance of acuteness of olfaction via the elimination of foreign materials including odorants**.** Given these factors which may explain the remarkable effect of malathion on the olfactory epithelium, in concert with the severity of the effect, as well as not knowing the time of onset of hyperplasia, I consider the application of a mere UF of 3 to cover for the lack of a NOEL to be entirely inadequate. I say this in view of both the smallness of the UF chosen, and an operating philosophy which in lieu of weighing the significance of the finding, simply invokes a UF without offering any explanation as to why 3 is adequate, or why another study should not be required. The April 27, 1995 HED memorandum conveying the DER to the Product Manager says among other things: "The question of carcinogenicity as it may relate to the microscopic lesions of the nose and larynx will be addressed in a separate memorandum." To my knowledge such a memorandum remains outstanding, and this very important issue has not been addressed.

D **Margin of Exposure for Occupational/Residential Exposures**

(1) MOE for Dermal Exposures: see comments as before on the use of reproduction and developmental toxicity studies to rule out the possibility of enhanced sensitivity of young animals.

(2) MOE for Inhalation Exposures: As stated above, I do not support the use of the UF of 3. Again I find unmerited the claim that:"No FQPA factors are required since there was no indication of increased sensitivity in the

offspring of rats or rabbits to prenatal exposure to malathion.", lacking cholinesterase data or behavioral effects assessments.

E **Recommendation for Aggregate Exposure Risk Assessments**
No additional comments

III. FQPA Considerations

1. **Neurotoxicity Data**
In the case of the acute neurotoxicity study, concerning bilateral retinal rosette observed in one male rat, the statement might be improved somewhat in its meaning by saying that the one rat in which it was observed was from among but five males examined histopathologically in the high dose group, and that none were examined in lower dose groups. Also, concerning the acute and subchronic neurotoxicity studies mentioned, I would cite my memorandum of November 20, 1997 to Clark Swentzel as detailing comments I might otherwise offer here.

2. Determination of Sensitivity
No further comments on the developmental and reproduction studies.

VII **Data Gap(s)**

Roman numerals go from III to VII in the HIDR.

From my perspective, the following are data gaps:

1. Carcinogenicity Study in B6C3F1 Mice (MRID 43407201) :
Pathology Working Group assessment for liver tumors;
Histopathology assessment of nasal tissues.

2. Combined Chronic Toxicity/Carcinogenicity F344 Rat Study (MRID 43942901) : Pathology evaluation/reevaluations of various tissues.

3. Subchronic Inhalation Study in Sprague-Dawley Rat (MRID 43266601) : resolution of no NOEL for nasal tissue histopathology, which was severe at the lowest dose and present in essentially all rats of both sexes; recommend a new and longer term study to address the absence of a NOEL and potential carcinogenicity by the inhalational route.

4. Developmental Toxicity Study in the Rabbit (MRID 152569) : submission of Appendix III followed by statistical treatment of the individual data to affirm the NOEL for body weight effects in dams particularly over days 6-12 of gestation.

5. Acute Neurotoxicity Study in the F344 Rat (MRID 43146701): submission of selected retinal tissue slides as called for in the DER.

6. Subchronic Neurotoxicity Study in the F344 Rat (MRID 43269501) : submission of a guideline behavioral test yet to be specified.

7. Three-month cholinesterase assay in the rat to determine a definitive LOEL/NOEL for malathion.

Brian Dementi, Ph.D, Toxicologist/HED

ATTACHMENT 7:Letter from B. Dementi - January 15, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

January 15, 1998

Re: Additional information concerninig the malathion acute neurotoxicity study.

One of the issues before the Hazard ID Committee in assessing the malathion data base is that of one incident of bilateral retinal rosette among five male rats in the high dose group in the acute neurotoxicity study. You will recall that the DER for that study calls for submission of selected histopathology slides for independent characterization of retinal rosette. I will not reiterate here that which has already been presented in earlier documents, but would like to introduce some additional information that may have some significance in the deliberative process.

Various published works indicate that the terms retinal rosette, retinal fold and retinal detachment may apply to the same or very similar anatomic or pathologic condition, all of which seem to involve a separation and folding of certain layers of the retina. This in itself is a complicated subject, and there may be varying terminologies for this phenomenon. [Tansley (1933); Lai and Rana (1985); Rubin (1874); Kuno et al (1991)] Furthermore, retinal rosettes are said to be rare in rats and are generally considered to be developmental effects, not occurring spontaneously in adult animals. According to Tansley (1933), an older but excellent reference, it is claimed that there is normally a difference in tension between the outer and inner layers of the ratina explained as follows: "That there is normally some difference in tension between the outer and inner parts, even in the adult retina, can be shown by removing it from the eye. If the tissues are still living, it will be found that the retina always curles up into a roll so that the rod and cone layer is on the inside." Further along the author says, "Under the conditions described in this paper there certainly seems to be a definite relation between the maintenance of a normal intra-ocular pressure and the appearance of retinal rosettes. In all the cases with which we have been dealing here the fact of a lowering or an absence of intra-ocular pressure also involves the dissociation of the retina from the wall of the eye to which it is normally attached during development" (p. 335) It is as if intra-ocular pressure helps sustain, physically, the retina in its normal contours. This particular publication involved studies on postnatal eye development in rats, but the implication is that even in the adult eye, intra-ocular pressure may be critical to the maintanance of retinal form.

Since organophosphate cholinesterase inhibitors have a medicinal use in the treatment of glaucoma, via reducing intra-ocular pressure, I decided to examine precautionary labeling on such medication. In the case of Ayerst Laboratories' Phospholine Iodide (echothiophate iodide for ophthalmic solution), an organophosphorothioate cholinesterase inhibitor, under Adverse Reactions, the first mentioned reads as follows, "Although the relationship, if any, of retinal detachment to the administration of Phospholine Iodide has not been established, retinal detachment has been reported in a few cases during the use of Phospholine Iodide in adult patients without a previous history of this disorder." Further, under Precautions, the seventh reads "Phospholine Iodide (echothiophate iodide) should be used with great caution, if at all, where there is a prior history of retinal detachment." It is also noteworthy that the echothiophate insert indicates that echothiophate potentiates other cholinesterase inhibitors such as organophosphate and carbamate insecticides, and that patients undergoing systemic anticholinesterase treatment should be warned of

the possible additive effects. A copy of the insert where these quotes may be found is appended.

In summary, this added information indicates that: 1) retinal rosettes, retinal folds and retinal detachments (even microretinal detachments) may be different terms for a common underlying effect; 2) maintanance of intra-ocular pressure may play an essential role in preserving retinal structure; 3) substantial declines in intra-ocular pressure could in principle elicit retinal detachment and/or scrolling of the retina; 4) organophosphate medicinals used to control intra-ocular pressure in the treatment of glaucoma, presumably when used with precise dosing under the care of a physician, have as an associated precaution retinal detachment; 5) malathion as evaluated in the acute neurotoxicity at single very high doses via oral gavage, in fact could have elicited a precipitous decline in intra-ocular pressure resulting in the retinal anomaly; 6) malaoxon, the active metabolite of malathion, like echothiophate is an organophosphorothioate. So whatever the mechanism of the possible association between treatment with echothiophate and retinal detachments in humans might be, that mechanism could in principle operate in the acute neurotoxicity study where very large doses were used.

Brian Dementi, Ph.D.
Toxicologist, HED

cc Jess Rowland

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ATTACHMENT 8: Letter from B. Dementi - February 10, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

February 10, 1998

In drafting the toxicology chapter of the Registration Eligibility Document (RED) for malathion and attendant closer evaluation of the Hazard ID Committee report for the November 6, 1997 meeting to consider malathion, there is yet another issue of concern that I believe merits resolution.

Under the topic of Reproductive Toxicity (pp. 15-16) of the Committee report, the following paragraph is found. "Although the offspring NOEL (131 mg/kg/day in males and 153 mg/kg/day in females) was lower than the parental systemic NOEL (394 mg/kg/day in males and 451 mg/kg/day in females), the Committee determined that this was not a true indication of increased sensitivity of offspring because: (I) pup body weight decrements were primarily observed at postnatal day 21; (ii) during that period (i.e., later portion of lactation), young rats consume approximately twice the diet per unit body weight as an adult rat consumes (i.e. 1 ppm in the diet of a young rat is approximately 0.1 mg/kg/day whereas in older rats, this ppm level is equal to 0.05 mg/kg/day) and (iii) the estimation of the test substance intake in pre-weaning animals is likely to be more than double the adult intake because of the availability of the test material both via the milk (lactation) and food, particularly after the mid point of lactation." While there is much that may be viewed as generally true in this statement, my concerns have to do with the reliability one can place in these arguments in this particular case, lacking definitive data, to conclude that offspring were no more sensitive than adults.

Although it is true that weight decrements were primarily observed at postnatal day 21, pup body weight decrements were statistically significant on days 7, 14 and 21 for the F2B generation at the penultimate dose level, which the study report itself concluded to be treatment related. No record is made in studies such as this of pup food consumption, so it is very presumptive in the particular case at hand to draw conclusions about what pups may or may not have consumed in the control and various dose groups. Generalities regarding relative food consumption of pups versus adults cannot be reasonably used to reach definitive conclusions pertaining to how much test material various dose groups may have been exposed to via the diet. Furthermore, there is no data in this study to show the presence or absence of malathion in the milk. There is an incumbency to have, or provide, data showing not only the presence of malathion in the milk, but how much is there, before concluding that malathion ingestion via the milk contributed in a sufficiently meaningful way to total intake and thus to support the argument that pup consumption on a body weight basis exceeded that of adults in this study. Hence, the reasoning used to dismiss the finding in this study of greater sensitivity of offspring are speculative, and certainly not of the definitive character required to refute the positive evidence that pups were in fact shown more sensitive than adults.

This particular evidence of increased sensitivity of offspring takes on peculiar importance in the assessment of malathion by the Hazard ID Committee in relation to the question of whether to remove the 10X factor required under FQPA for infants and children. The Committee concluded that the 10X factor should be removed in part because: " A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults." (p. 18) The Food Quality Protection Act (1996) requires use of an extra 10-fold safety factor in addition to the traditional 100-fold safety factor, unless, on the basis of reliable (emphasis added) data, a different level is

determined to be safe for children. It is my understanding that the intent of Congress, as reflected in FQPA, was to afford additional protection for infants and children unless and until it can be reliably shown that the 10X or a lesser added factor is not needed. The burden of proof rests with the Agency to show via the absence of any evidence of increased sensitivity of offspring, and in the face of a complete data base, that the added factor is not necessary. As I endeavored to persuade the Committee in my December 17, 1997 comments addressed to Jess Rowland, developmental and reproductive toxicity studies are inherently weak to detect the more subtle effects of cholinesterase inhibition, indeed such studies do not even incorporate cholinesterase assessments, therefore there is enhanced reason to rely on parameters such as body weight, crude as they may be, as evidence of effects of a test material. If cholinesterease data in offspring and that versus adults were available in this study, less reliance would need to be placed on the body weight data. But given the situtation as it exists, I consider the reasoning used by the Committee to dismiss evidence of enhanced sensitivity in offspring in the two generation reproduction study to violate the intent of Congress to the end that the 10X factor be discounted only on the basis of reliable data.

Brian Dementi, Ph.D.
Toxicologist, HED

cc Jess Rowland

ATTACHMENT 9: Letter from B. Dementi - March 10, 1998

Jess Rowland, Secretary
Hazard ID Committee

March 10, 1998

This is an addendum to my December 17, 1997 comments to you on the Hazard ID Committee report for the November 6, 1997 meeting on malathion. My comments here pertain to the subchronic inhalation study. I recently requested from the registrant's representative a copy of the range-finding inhalation study. The study is entitled "A 2-Week Toxicity Study of Aerosolized Malathion Administered by Whole-body Inhalation Exposure to the Albino Rat" completed on July 20, 1993. Concentrations evaluated in this study were 0, 0.56, 1.58 and 4.23 mg/L, as contrasted with those employed in the full subchronic study of 0, 0.1, 0.45 and 2.01mg/L. After two weeks of treatment, with respect to upper respiratory findings, the Summary of the study claims that histological findings on the nasal and laryngeal mucosa were observed in most low dose animals and in the majority of the mid and high dose animals. "These findings included a slight to mild loss of goblet cells and similar hyperplasia in the nasal respiratory epithelium, slight leucocyte exocytosis in the nasal squamous and respiratory epithelium and slight to mild epithelial hyperplasia of the laryngeal mucosa." (p. 10) The fact that there was no NOEL for nasal and laryngeal effects after only two weeks of exposure demonstrates a much earlier onset of the nasal effects than could be determined from the subchronic inhalation study with malathion or the chronic feeding studies with malathion and malaoxon, where similar nasal and laryngeal effects were observed.

These histopathologic findings, without a NOEL, in this range-finding study after only two weeks of exposure, taken together with similar findings in the other longer term studies, serve to reinforce my opinion that another inhalation study is needed to identify a NOEL, and to determine the time of onset and ultimate course for nasal and laryngeal effects. Again, I consider inadequate the Hazard ID Committee's decision to employ a UF of 3 to compensate for the absent NOEL for this effect in the subchronic inhalation study. Your February 1997 Guidance Document for the Toxicology Endpoint Selection Process claims that "However, a LOEL may be used if a NOEL is not established in the critical study, when severity of the effects observed at this dose is of negligible concern for human risk, or when there is a data gap. Therefore, when a LOEL is identified for risk assessment, additional modifying factors (range of 3 to 10) may be used in addition to the total Uncertainty Factorof 100 (i.e., 10 for intra- and 10 for inter-species variation)." (p. 12) In response to this, I cannot accept the premise that the severity of the nasal and laryngeal tissue effects are to be viewed as of such "negligible" concern for human risk as to justify use of a modifying factor as explained in your paper. Furthermore, if the committee were inclined on employing a modifying factor of between 3 and 10, what reasoning was invoked to support choosing the low factor? Please be reminded that at the Cancer Assessment Review Committee meeting of last September-October these nasal tissue findings in the chronic feeding studies were considered of sufficient concern as to require additional nasal histopathology in the malathion rat and mouse studies.

Brian Dementi, Ph.D.
Toxicologist/HED

ATTACHMENT 10: Letter from B. Dementi - March 16, 1998

Jess Rowland, Secretary
Hazard ID Committee

March 16, 1998

This is a further addendum to my December 17, 1997 comments to you on the Hazard ID Committee report for the November 6, 1997 meeting on malathion. My comments concern a 2-week range-finding inhalation study which I mentioned to you in my March 10, 1998 memorandum. In that earlier communication I commented on histopathology findings in nasal and laryngeal tissues. In this case I would like to advise that in the range-finding study involving test concentrations of 0, 0.56, 1.58 and 4.23 mg/L, a NOEL was not identified for erythrocyte cholinesterase inhibition in either sex, or for plasma or brain cholinesterase inhibition in females. In males, the NOELs for plasma and brain cholinesterase inhibition were 0.56 mg/L and 1.58 mg/L, respectively. The number of rats under test in this study was but 5/sex/group (in certain groups the number composing mean values for cholinesterase activity were less than five due to various reasons given in tables of individual data), and there was no statistical treatment of the cholinesterase data in the study report as submitted. So the reported findings cannot be considered definitive, as is often characteristic of range-finding studies. However, it should be noted that in terms of percent enzyme inhibition relative to controls, after two-weeks of exposure in this range-finding study, cholinesterase inhibition (plasma, erythrocyte and brain) is reasonably complementary with that of the full subchronic inhalation study after 90-days of treatment. To the extent that the data may be considered reliable, there is little evidence of a cumulative effect of malathion over 13 weeks as opposed to 2 weeks. The two studies taken together (see attached table) yield reasonably consistent dose-reaponse data across the overall dose range of 0.1 to 4.23 mg/L, with the data indicating that erythrocyte cholinesterase is virtually equally responsive in both sexes, but that females are more remarkably affected in terms of plasma and brain cholinestrerase inhibition. The range-finding data also tends to strengthen the conclusion in the subchronic study that there is no NOEL for plasma cholinesterase inhibition in females and possibly for erythrocyte cholinesterase in both sexes. Overall, the data indicate females to be the more sensitive gender.

Brian Dementi, Ph.D.
Toxicologist/HED

Comparative Cholinesterase Inhibition in 2-Week Range-Finding and 90-Day Subchronic Inhalation Studies in Sprague-Dawley Rats

	Atmospheric Concentrations (mg/L)					
	0.1	0.45	**0.56**	**1.58**	2.01	**4.23**
Enzyme Inhibition,%						
Plasma Cholinesterase						
Males:	2	7	7	20	18	50
Females:	16	30	49	71	70	84
Erythrocyte Cholinesterase						
Males:	9	22	18	33	43	58
Females:	11	27	26	39	44	53
Brain Cholinesterase						
Males:	5	3	0	4	17	36
Females:	4	8	12	18	41	59

Notes: Bold print atmospheric concentrations from 2-week range-finding inhalation study (5 rats/sex/group); Normal print concentrations from subchronic (90-day) inhalation study at term (15 rats/sex/group).

ATTACHMENT 11: Letter from B.Dementi - March 20, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

March 20, 1998

Re: Acceptability of the malathion chronic dog study by the Hazard ID Committee; recommendation for additional testing of cholinesterase inhibition in dogs.

The following comments are intended to summarize the historical record regarding the status of the acceptability of the malathion one-year chronic dog study (MRID 40188501), and to present rationale in support of additional testing for cholinesterase inhibition in the dog.

The October 5, 1987 Data Evaluation Record (DER) concluded the chronic dog study to be *Supplementary*, attributable to the lack of NOELs on several toxicology end points. The October 7, 1987 covering memorandum addressed to the Product Manager called the study *Supplementary* and claimed it would not serve to satisfy guideline requirements. When the study was taken under advisement by the TOX-SAC (report date: September 11, 1997), that committee concluded the study to be *Unacceptable*. In accordance with that conclusion, the DER was subsequently revised in preparation for the November 6 Hazard ID Committee presentation. The revised DER claimed that: "This study is **NOT ACCEPTABLE** (Supplementary) and **DOES NOT SATISFY** guideline 83-1 for a chronic toxicity study in dogs because NOELs were not established for inhibition of cholinesterase activity for plasma and erythrocytes in either males or females." I interpret this to mean that among the several NOELs cited in the original DER, the TOX-SAC narrowed the findings of concern down to cholinesterase inhibition as the basis for claiming the study to be *Unacceptable*. This was the assessment prior to the Hazard ID Committee meeting.

Having examined the December 17, 1997 report for the Hazard ID Committee meeting, I find no specific reference to any deliberations regarding the status of acceptability of the study, nor any statement to the effect that the committee reversed the conclusions of the TOX-SAC. In recent consultation with the toxicologist who presented the data base to the Hazard ID Committee, he speaks of having no recollection of raising the issue; nor do I recall its being mentioned, though perhaps it was so mentioned in passing. The December 17 report does say there are no data gaps, but there is the question as to whether that statement refers to the entire data base, or more narrowly to those studies that would address relative sensitivities of developing and young individuals to adults for purposes of deciding the need for the FQPA imposed 10X safety factor to protect infants and children. To the extent that the "no data gaps" statement refers to the entire data base, we know that is incorrect because of the unresolved malathion carcinogenicity issues identified at the September-October 1997 Cancer Assessment Review Committee meeting. I believe in order to set the record straight, the report of the Hazard ID Committee meeting should convey in unambiguous terms that the committee did not concur with the TOX-SAC, if that is indeed what transpired..

As relevant to this question, your committee should be advised that a memorandum was written on March 26, 1990 by B. Dementi to P. Fenner-Crisp, then director of HED, conveying the results of a March 1, 1990 Cholinesterase Peer Review Committee meeting in which the status of the acceptability of the dog study was reviewed. That committee concluded that the study would satisfy the section 83-1 data requirement. (Attachment 1) I doubt that this memorandum was among documents reviewed by either the TOX-SAC or the Hazard ID Committee. Presumably

the results of that 1990 peer review were communicated to the Product Manager, and thence to the registrant. The March 27, 1997 malathion oneliners lists the study as Supplementary without offering any qualification.

Given this background, I consider it necessary to provide additional perspective on this subject. In April of 1992, I drafted a memorandum addressed to the Product Manager recommending further study in the dog. That draft memorandum was signed by me on April 16, 1992. (Attachment 2). The reasoning for that recommendation, as set forth in the memorandum, was in part to help determine whether the dog or the rat should be selected as the preferred surrogate species for humans in ocular effects testing [see Exhibit I (p. 5) of my November 10, 1997 letter to you]. Ocular effects of organophosphates had become an important topic at that time, and remains so today. The April memo also claimed the work was needed to identify the NOEL for cholinesterase inhibition in the dog, as it was not achieved in the subject study. I must stand behind that recommendation today.

Supporting this recommendation, it may now be said, is the responsibility to obtain **reliable** (a word judiciously employed under FQPA) assessments of cholinesterase inhibition in the dog. I would note the following. In the February 1988 Malathion Registration Standard (OMB Control No. 2070-0057), the guideline requirement for a 90-Day (Subchronic) Feeding Study in the dog was waived: "...since requirments for chronic rodent and non-rodent toxicity studies have been imposed." (p. 123) Since the subchronic study was waived, there is to that extent, in my judgment, a greater burden to have in place a fully acceptable chronic study. The subchronic study would have been a feeding study which could have helped addressed the question of whether the method of dosing (oral capsule) in the chronic study compromised the expression of cholinesterase inhibition, and perhaps whether the dog is as refractory to malathion induced cholinesterase inhibition, as some seem willing to accept. Indeed, in my opinion the obtaining of an acceptable chronic dog study is implicit in the waiver of the subchronic study. I recently examined the malathion one-liners for dog feeding studies, and found none. So other than the one chronic study, there is a paucity of relevant data on dogs.

There are yet additional reasons why **reliable** cholinesterase data should be in place with respect to the dog: 1) The FIFRA guidelines intend that acceptable data be in place on multiple species since animal models are used as surrogates for human responses. It should be viewed as especially important in this particular case with malathion, because **reliable** data on the dog is not available, in the face of a remarkable contrast between the sensitivities of human and rat, i.e. definitive data on a third species is indicated; 2) As is true in the case of humans, the dog lacks carboxylesterase in the plasma [see Exhibit 1 (pp. 3-5) in my November 10, 1997 to you], which in principle should render the dog (like the human) more susceptible than the rat to the cholinesterase inhibiting effects of malathion; 3) I recently compared the dog and rat studies for a few organophosphates in the Caswell file and found the dog to be very responsive, exhibiting no such remarkable differences versus the rat as is evident in the malathion case; 4) Since the dog study was reviewed, serious questions have arisen within HED as to the adequacy of cholinesterase methodology employed in data submissions in general. I have participated in workshops on cholinesterase methodology. In my view this is a serious matter with respect to the degree of **reliance** to be accorded data submissions, particularly those such as the malathion chronic dog study where there is considerable puzzlement over the apparent lack of responsiveness and no adequate explanation. Further, I remain uncertain at this time as to the final outcome of in-house assessments pertaining to the grander question of the adequacy of cholinesterase methodology, and what may have been recommended in more recent times that would assure proper assessment of cholinesterase inhibition. In retrospect, I now believe the 1990 Cholinesterase Committee erred in assuming that because the dog yielded a weak response in the chronic study that the NOEL, once properly determined, would be far above that in the rat and human. Such a position accepts uncritically that cholinesterase methodology employed in the study was satisfactory. Furthermore that view neglects to recognizer that the response could may be entirely different in a feeding study, such as the one waived.

The bottom line to all this is that there exists a data gap for cholinesterase inhibition in the dog. In my view, an additional malathion study should be required to allay concerns over the questionable data now in place. This is a particularly important requirement since the RfD is based on cholinesterase inhibition, and the Hazard ID Committee has shifted the defining study from that of Moeller and Rider (1962) in humans to that of the recently submitted chronic toxicity/carcinogenicity study in the rat, which study has an anomaly at the lowest dose for erythrocyte cholinesterase inhibition in females, as I have noted in my December 17, 1997 comments to Jess Rowland, and presented more fully in my November 10, 1997 letter to you. I consider it unfortunate if the registrant has been advised that no additional work in the dog is necessary. However, perspectives have changed since the 1990 peer review, and the public interest is of far greater moment than the additional effort this requirement would entail.

Sincerely,

Brian Dementi, Ph.D.
Toxicologist/HED

cc Jess Rowland

ATTACHMENT 12: Letter from B. Dementi - July 27, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

July 27, 1998

Re: Malathion External Peer Reviews

As you know, following the November 6, 1997 Hazard ID Committee meeting on malathion, I drafted a number of letters in response to the minutes, directed either to you or Jess Rowland. As an approach to addressing my questions, the Office elected to invite comments from external experts in toxicology. In preparing for the external peer review, I drafted a set of questions, numbered I-VIII, accompanied by pertinent reference materials, which were provided to the reviewers via OPP's external peer review coordinator, Dr. Hank Spencer. OPP introduced, preliminary to my set of specific questions, a "Charge to the Reviewers" which called for quality assessments of the various DERs in general, and whether the appropriate uncertainty factor was used for the RfD.

The external reviewers, Drs. Michael Dourson, Rolf Hartung and Walter Decker, have now provided their responses. Their letters are expected to be included in the package of documents to be considered by the Hazard ID Committee at its August 18 meeting. I would like to request that you provide Committee members a copy of the entire package, including all referenced materials, that was available to the external reviewers. This represents considerably more information than was presented on November 6. I would hope the Committee might have adequate time, possibly a little more than usual, to study the package. I would be available in the interim to respond to any questions or comments anyone wishes to pose.

In an effort to compare and interpret the reviewers' responses to my questions, I have consolidated, in the format of the same questions, abbreviated conclusions of each reviewer under each question, in order to view juxtaposed the responses to assess the level of concurrence and the extent to which they, collectively, have helped me and hopefully others in understanding the facts before us. The abbreviated conclusions represent my best judgement of what they communicated as gleaned from their more detailed responses. So I would urge Committee members to confirm whether my interpretations are appropriate.

Also, under each question, I have included comments which represent my effort to estimate not only how well the reviewers agree, but whether and to what extent they have guided me in addressing my questions. This *Consolidation of External Peer Reviewers' Comments on Malathion Non-Cancer Issues* dated July 27, 1998, written by me, is appended to this memorandum.

Brian Dementi, Ph.D. DABT
Toxicologist
Toxicology Branch I/HED

CONSOLIDATION of EXTERNAL PEER REVIEWER'S COMMENTS on MALATHION NON-CANCER ISSUES. by BRIAN DEMENTI JULY 21, 1998

I Hazard Identification/Acute Oral (One-Day)

Supporting documentation: DER #s 5, 6, 7, 9 and 19; References: A (pp. 3-5), B (pp.1-4), C, D, E , V and BB (pp. 12-14; 20-22)

Question **1): Do the rabbit developmenal toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicologic consequence in either the maternal or the developing organisms?**

Dr. Dourson: No.

Dr. Hartung: No.

Dr. Decker: No.

Comments: The external reviewers do not accept that a single dose as high as 50 mg/kg would be without toxicologic effect in maternal or developing organisms based on the rabbit developmental toxicity studies.

Question 2)**: Does data on maternal body weight and body weight gain now available in Appendix III of the rabbit developmental toxicity study alter the assignment of the LOEL/NOEL for the study, and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxicologic effect?**

Dr. Dourson: No.

Dr. Hartung: No.

Dr. Decker: No.

Comments: The external reviewers agree that data in Appendix III would not influence the conclusion. We should note that data in this appendix has not been analyzed, statistically, in HED.

Question 3): **As presented in a published work in the open literature, single intraperitoneal doses as low as 50 mg/kg in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterases were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg can serve as the endpoint for acute dietary (one-day) risk assessment?; c) Is the data available in the developmental toxicity studies sufficiently reliable to discount the 10X safety factor required under FQPA?**

Dr. Dourson: Says the study has advantage of testing a relevant effect. Route of exposure is an issue. "I am not satisfied that potential risks to humans is addressed with the data available in this review package. But more data are probably available to further address this question. A discussion of uncertainty factors for potential data base gaps should be postponed pending the review of these additional data." (p. 4)

Dr. Hartung: Says behavioral effects that have a degree of correspondance with cholinesterase inhibition are to be expected, but there is no requirement that dose response curves for both to coincide. Intraperitoneal route is of questionable surrogacy for realistic exposures. Says data does not support deletion of the 10X factor.

Dr. Decker: Accord low level of confidence to the study because i.p. cannot directly compare to real exposures. Says cannot dismiss the 10X factor

Comments: The external reviewers consider the study to be of value in that it assesses relevant effects, and supports a degree of correspondance between cholinesterase inhibition and behavioral effects, but all appear to agree that data from developmental toxicity studies, and perhaps the entire malathion data base, does not support deletion of the 10X safety factor imposed by FQPA. My principal reason for citing Kurtz (Ref. D) was to illustrate that a single dose at 50 mg/kg can elicit a remarkable response. Furthermore, the study shows that at doses extending below those inhibiting cholinesterase, a behavioral effect has been observed, even if the route of administration differs from that of normal human exposure. None of the reviewers question the quality of the study, or the validity of the findings.

II Determination of Susceptability, Reproductive Toxicity

Supporting documentation: DER: # 5; References: A (pp. 15-16), B (pp. 3-4), F, G and BB (pp. 12-14; 16-17; 20-22)

Question 1) **Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rat be dismissed as "...not a true indication of increased sensitivity of offspring..." for the reasons stated in the Hazard ID Committee report?**

Dr. Dourson: Yes, to the extent that the dose in offspring is not derived from actual assessment of food intake.

Dr. Hartung: Yes, but expresses the view that neonates must be shown to be less sensitive than adults (not equal to) before the FQPA 10X safety factor can be deleted.

Dr. Decker: No, "because some toxic effects have been reported."

Comments: Two reviewers say yes (with qualifying remarks) and one says no. I had hoped the reviewers would say something specific about views expressed in Ref. F, supported by data in Ref. G (selected pages from the study report). The point is that an effect on pup body weight occurred at a dose below that which similarly affected dam body weight. The effect on pups was dismissed by the Hazard ID Committee as evidence of greater sensitivity of pups for reasons which in my view were unsubstantiated, i.e. no proof of the presence of malathion in the milk, nor any evidence of how much food pups may have consumed under circumstances wherein malathion in the diet may have influenced food intake. It may not have been clear to

the external reviewers that the presence (let alone the amount) of malathion in the milk has not been shown by analysis. It should also be noted that while pup body weight changes were seen during lactation days 7 (where pups rely essentially exclusively on milk), 14 and 21 in the 5000 ppm dose group (the NOEL for dam body weight change in the study at large), dam body weight changes were not apparent during the lactation period even at the top dose of 7500 ppm. Hence, during lactation pup NOEL/LOEL = 1700/5000 ppm, while dam NOEL ≥ 7500 ppm (HDT). Pope and Chakraborti (1992) (Ref. E) say that young mammals are remarkably more sensitive than adults to numerous organophosphates. Hence, the burden is not light to justify dismissing evidence of a more selective effect in pups due to exposure to this particular OP.

Question 2) **In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are the more sensitive to malathion?**

Dr. Dourson: No.

Dr. Hartung: Seems to say no since the data in question do not exist. Though at this point he does not actually affirm the critical importance of the data in question, he attests to the importance elsewhere in the document. For example, in defending the use of the human cholinesterase study, Moeller and Rider, he says: "....it addresses a diagnostic end-point that is known to be mechanistically related to the toxicity of OPs." (p. 8); and "Changes in some behavioral parameters that have a degree of correspondance to acetylcholinesterase, in particular to brain cholinesterase, would be expected." (p. 5)

Dr. Decker: No. Says more behavioral (learning) tests should be performed. FIFRA Guidelines need updating.

Comments: The external reviewers appear to agree in saying no to this question, i.e. data in the 2-generation reproduction study are not adequate to address the question of relative sensitivity of young versus mature animals.

Question 3) **Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10X safety factor imposed by FQPA as additional protection for infants and children?**

Dr. Dourson: Suggests 3X as opposed to 10X safety factor. Although, he acknowledges 10X may still be useful as a management tool.

Dr. Hartung: No. Expresses view that the study shows no clear evidence of less sensitivity of offspring, which he considers essential.

Dr. Decker: No. "....evidence seems quite thin." (p. 5)

Comments: The weight of opinion is that the 10X safety factor under FQPA cannot be dismissed.

III Hazard Identification/Chronic Dietary (RfD)

Supporting documentation: DERs: #s 1 and 10; References: A (pp. 5-6), B (pp. 4-5), H, I, N (p. 16), R and Y.

Question 1) **Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?**

Dr. Dourson: Yes, but recommends an additional 3-fold uncertainty factor be applied to the NOEL in the rat in establishing the new RfD , as indicated in question 5.

Dr. Hartung: No.

Dr. Decker: No.

Comments: Dr. Dourson says yes to this question, but it is not clear what his opinion would be in the event an additional uncertainty factor were not used with the rat data as he proposes. The other two reviewers agree that it cannot be said that 50 ppm was a NOEL in view of the findings in the background papers. Elsewhere in their comments, Dr. Hartung says: "I find the discussion regarding the selection of plasma cholinesterase inhibition for the determination of the RfD to be simplistic and superficial." (p. 3) Dr. Decker says with regard to the question of whether the human or rat data should be used for establishing the RfD: "I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in data gaps." (p. 4) Dr. Decker thus acknowledges data gaps. He also says: "I am not aware of supporting studies which shore up the use of the principal study for the RfD." (p. 4) It is reasonable therefore to conclude that a consesus exists that the study does not satisfactorily identify a NOEL for cholinesterase inhibition. It should be noted that the registrant was advised before conducting the chronic toxicity/carcinogenicity study in the rat that 100 ppm would be expected to be an effect level for cholinesterase inhibition (Ref. I) Three months is an important time period, as within this time frame important adjustments to the treatment may occur.

Question 2) **Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?**

Dr. Dourson: No comment on cholinesterase methodology.

Dr. Hartung: Says requires analysis of detailed cholinesterase methodology.

Dr. Decker: Says this is a possibility, and if so, concern extends to all OP pesticides.

Comments: This question was posed primarily because erythrocyte cholinesterase was clearly inhibited in females at the 100 ppm and 500 ppm dose level after three months of dosing, but not at 50 ppm or 500 ppm at six months. These contrasting findings at 500 ppm cloud the interpretation as to whether 50 ppm would have been an effect at three months had it been tested. In the views of the external reviewers, it would appear to be an outstanding question that requires resolution. Perhaps results of OPP's workshops on cholinesterase methodology could help resolve this question.

Question 3) **Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in males, be supported as a**

replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?

Dr. Dourson: Yes to the first part of question. Says additional testing not needed. Suggests benchmark dose analysis in event some scientists wish to pursue whether 50 ppm is a NOEL in females. Notes that 50 ppm was a NOAEL in the 13-week neurotoxicity study. However, recommends additional 3-fold uncertainty factor as indicated in Question 5.

Dr. Hartung: No to the first part of question, and is critical about replacing human data with animal data.

Dr. Decker: No to the first part of question. Recommends additional testing to identify NOEL in rats of both sexes.

Comments: Same as those under question # 1. In addition I should reference my concerns about placing reliance upon the NOEL for cholinesterase inhibition in the 13-week neurotoxicity study as expressed in Ref. B (pp. 4-5).

Question 4) **Given that an explanation exists for a greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e. the lack of carboxylesterase in human plasma) should a 10X safety factor applied to the rat data to allow for "uncertainties" in interspecies variability be considered adequate if the rat data is to be used in deriving the RfD?**

Dr. Dourson: Yes, but advocates an additional 3-fold uncertainty factor for other reasons as indicated in question 5.

Dr. Hartung: No

Dr. Decker: No, but would be acceptable with enhanced testing in the rat.

Comments: The reviewers' comments are important in underscoring the fact that the data base is inadequate as it stands in establishing an RfD. Actually, in posing this question, I was seeking the reviewers' opinions as to whether the concept of using a 10-fold safety factor intended to account for uncertainties in interspecies variability is adequate in the face of known differences in sensitivity. Stated differently, should corrections to accomodate know differences, which may even exceed 10-fold, first be introduced, followed by the 10-fold factor to address the unknown species differences in susceptability? (Ref. I) It is not clear to me that this particular philosophical question was recognized or responded to, but remains a question for the Hazard ID Committee.

Question 5) **Further, given that the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?**

Dr. Dourson: No, but advocates an additional 3-fold uncertainty factor to account for deficiencies in the data base, principally because the critical effect (cholinesterase inhibition) was not monitored in the 2-generation reproduction study in a potentially sensitive subgroup (i.e. young rats), which he characterizes as a

data gap (p. 3). Also, suggests an added uncertainty factor of unspecified magnitude, probably less than 3 in his view, for the RfD based on the human study, should it be retained, since females (women) were not tested.

Dr. Hartung: Yes.

Dr. Decker: Yes.

Comments: Given that Drs. Hartung and Decker say, emphatically, the human study should be retained, and Dr Dourson does not provided an unqualified differing opinion, a consesus exists that the human study should be retained. If it is to be retained, an added safety factor should be considered based upon Dr. Dourson's comments..

Question 6) **Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?**

Dr. Dourson: Does not answer the question as such, but acknowledges in Question IV, # 5 recognition the study does not test toxicity in young rats, and, hence, lacks surrogacy for infants and children. He asserts that the FQPA safety factor should not be considered in a discussion of science. He discusses his interpretation of the FQPA 10X factor as a safety factor for use in risk management toward the protection of infants and children, as opposed to that of an uncertainty factor.

Dr. Hartung: No, since the available information does not support the hypothesis that neonates are less sensitive than adults (see his p. 6)

Dr. Decker: No.

Comments: In disagreeing with the context of the use of the 10X safety factor, Dr. Dourson in my view did not respond with an opinion as to whether this study in any way supports discounting imposing the factor. Drs. Hartung and Decker say no. It would appear reasonable to conclude the reviewers feel the study does not provide any support for discounting use of the safety factor.

IV Subchronic Inhalation Study

Supporting documentation: DERs: #s 1, 2 and 13; References: A (pp. 9-11), B (pp. 5-6), J, N (p. 12) and O. ***(Note to Hazard ID Committee: please also see Ref. CC. This reference was in the package submitted to the external reviewers, but was not listed here among supporting documents for this question.)***

Question 1) **Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngal degeneration/hyperplasia supportable?**

Dr. Dourson: No. Advocates use of 10X rather than 3X uncertainty factor.

Dr. Hartung: No. Questions inhalation test procedure (whole body). Says finetuning (i.e., interpreted to mean use of 3X, or other factor) cannot accommodate gross deficiencies.

Dr. Decker: Says does not understand derivation of 3X uncertainty factor.

Comments: Given the inability for Dr. Decker to respond, taken in concert with the negative responses of Drs. Dourson and Hartung, the consesus of the external reviewers is that use of a mere 3X uncertainty factor is inadequate.

Question 2) **A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not identify NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to envoke an uncertainty factor for acute risk assessment (i.e. 1-7 days) on the basis of cumulative effects?**

Dr. Dourson: Yes (implied). Presents the argument that comparative findings in the 2-week and 90-day studies do not support a very remarkable cumulative response, and thereby, perhaps unwittingly, dismantles the Hazard ID Committee's principal argument for not invoking the uncertainty factor in the case of short-term exposures.

Dr. Hartung: No. Same comment as in question 1

Dr. Decker: Says a rangefinding study should not be used to decide, since such studies do not provide reliable information.

*Comments: Given the nature of responses from all three reviewers, I believe the question was not particularly clear. The Hazard ID Committee advocated a 3X uncertainty factor for the intermediate and long-term, but not for short-term(1-7 days) exposure risk assessments. The decision for not invoking the factor for the short-term exposures was predicated on the assumption that the end points in question identified in the 90-day inhalation study were cumulative in nature, and would not likely occur following the shorter term exposures. However, upon retrieving the 2-week rangefinding inhalation study, which was not available to the Hazard ID Committee at the November 6 meeting, it became clear that cholinesterase inhibition and, particularly, nasal and laryngeal hyperplasia were evident after only two weeks, and thus the argument for not applying the uncertainty factor for short-term exposures could no longer be supported. (See Refs. O and **CC**) Indeed, Dr. Dourson expresses the view that the end points in question may not be particularly cumulative based upon similarities of responses in the 2-week and 90-day studies. I generally agree with Dr. Decker that range-finding studies perhaps do no often provide reliable information, but in this case the range-finding study is of higher quality than most such studies, and I believe to be suitable to the extent of revealing early onset of the nasal tissue effects, and cholinesterase inhibition. So while the reviewers did not clearly address the question as to whether the uncertainty factor should be used in the case of the short term (1-7 days) exposures, the question stands, begging a response from the Hazard ID Committee.*

Question 3) **Should another study be required to identify the NOEL for the end points in question?**

Dr. Dourson: Yes (qualified). Suggests <u>first</u> using bench dose approach.

Dr. Hartung: "Not with rats on these issues." (p. 9)

Dr. Decker: Yes. "Common sense dictates that NOELs be identified." (p. 6)

Comments: Dr. Dourson evidently recognizes the need to more fully characterize the responses, i.e. a deficiency exists as it currently stands. Perhaps someone expert in this area could be commissioned to perform the tasks he suggests, and lets see what it shows. Dr. Hartung questions the utility of the inhalation study. However, the Agency requires the study and it is necessary that we assess the results. Dr. Decker most clearly enunciates what should be the Agency's position, which is to identify the NOELs on this very important end point for a very important route of exposure. It should be noted that in DER # 1 an extensive discussion is presented, indicating the very remarkable metabolic capability of the nasal olfactory epithelium and includes discussion as to why malathion may be a good candidate chemical to elicit nasal effects following metabolic conversion by the nasal tissues.

Question 4) **Given the findings of nasal and laryngal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalational route (e.g., inhalation exposure for first 90 days of a two year study)?**

Dr. Dourson: Yes (qualified). As in his response to the previous question, he says first ask for mechanistic studies to understand nasal injury. Use extrapolation via cancer guidelines.

Dr. Hartung: No answer. Still questions utility of inhalation studies.

Dr. Decker: Yes.

Comments: Dr. Dourson recognizes the need to address the issue, but proposes as a first alternative pursuit of mechanistic studies and extrapolation techniques. Perhaps someone expert in this area should be assigned the task and lets see what it shows, but I am not certain the most critical mechanism is identifiable with any certainty. Actual testing may be the best and perhaps only way to obtain satisfactory results. Dr. Decker is clear in his response that the study should be pursued. At other places in his response, Dr. Decker says: "The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups." (p. 2) We should note one of the rats in question had a carcinoma while the other had an adenoma of the olfactory epithelium. Were his suggestion to be followed, the inhalational route of exposure may be preferred, particularly if the study could be conducted in a manner acceptable to Dr. Hartung.

Question 5) **Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?**

Dr. Dourson: No. Acknowledges study does not evaluate young individuals. Asserts the FQPA 10X factor to be a risk management tool.

Dr. Hartung: No.

Dr. Decker: No.

Comments: The external reviewers agree the study does not provide any support for discounting use of the 10X safety factor imposed under FQPA.

v Acute Neurotoxicity Study (Retinal Rosettes)

Supporting documentation: DER #s 9 and 10; References: L, M and P (pp. 1-2)

Question 1) **Should retinal histopathology data be submitted for rats in the intermediate dose group?**

Dr. Dourson: Suggests first requesting submission of slides in question and then decide whether to evaluate lower dose groups.

Dr. Hartung: Yes

Dr. Decker: Yes

Question 2) **Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparison between control and treatment group lesion) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?**

Dr. Dourson: Yes

Dr. Hartung: Yes (evaluate the matter by either approach)

Dr. Decker: Yes

Comments: All three reviewers share an opinion that additional work is indicated, the question is whether the work called for in both questions should be pursued. Dr. Decker says yes to both, while Dr. Dourson suggest that examining lower dose groups would be contingent upon the results of the independent histopathology examination proposed. Dr. Hartung advocates additional work to resolve the question. If it cannot be determined by the Agency's pathologist(s) whether the retinal finding in the high dose male group is dosing related, then it is important to acknowledge that the Guidelines require examination of lower dose groups.

VI Subchronic Neurotoxicity Study

Supporting documentation: DER #s 10, 11; References: D, P (pp. 3-4), Q, S, T, U and BB (pp. 12-14; 16-17; 20-22)

Question 1) **Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity end points in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study or another neurotoxicity study that embraces learning/memory, EEG, EMG , and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?**

Dr. Dourson: No. His reason resides in an opinion that if the study were performed, it would not likely yield a result that would infringe the RfD.

Dr. Hartung: Yes (implied), but questions the acceptability of Russian neurophysiology (EEG, EMG) assessments.

Dr. Decker: Yes

Comments: Dr. Dourson says no to this question for the reason that the LOEL of 38 mg/kg/day is not inconsistent with the cholinesterase NOEL in the 2-year rat study (a noteworthy observation in itself, attesting to the credibility of the non-Guideline study). He proposes applying a safety factor to the LOEL, which raises a concern analogous to that in the case of the inhalation study (Question IV), as to whether that is a suitable approach for these end points. The problems I find with this are: 1) the identification of an end point to be used for regulatory purposes, in this case the RfD based on cholinesterase inhibition, should be selected in light of what the collection of Guideline studies reveal, i.e. all Guideline testing requirements should be satisfied, ideally each having been pursued to the point of rational conclusion. Each type of study in the Guidelines has its purpose; 2) Behavioral effects are of the highest order of importance; 3) If indeed the findings in Desi et al should be corroborated to show that behavioral effects, effects on neurophysiological parameters (e.g. EEG, EMG) and cholinesterase inhibition occur in neurotoxicity studies at doses comparable to those of cholinesterase inhibition in the Guideline 2-year rat study, the RfD derived from the latter would then have enhanced meaning among those persons who argue that cholinesterease inhibition itself, in the absence of other effects, is of questionable concern; 4) The Desi et al study did not identify NOELs on the very important parameters mentioned, and more than speculation should be employed to say at what doses effects terminate; 5) Desi et al was conducted in the female rat, and a question remains whether the Guideline 2-year rat study identified a NOEL for erythrocyte cholinesterase inhibition in the female rat.

Dr. Hartung says, prior to answering this specific question: "The assessment needs to incorporate the entire harmonized data set from all studies. It should not depend upon a search for single values, which are then treated without context." (p. 3) He also says: "It would be desirable to have at least a brief discussion of the interrelations of the various cholinesterases at different sites, their functions, and their diagnostic utility in relation to OP poisoning." (p. 4) This is a tall order as we all know, and this is why the implications of studies such as Desi et al indicating correlations between cholinesterase inhibition and other effects at low doses should not be dismissed out of hand. I am puzzled by certain elements of his response to the question at hand. He says: "The studies in DER #10 and DER #11 show no behavioral effects at dose levels significantly above dose levels associated with plasma cholinesterase inhibition, but they do show abnormalities in EEG and EMG recordings after 90 days of exposure." (p. 10) Actually, in Desi et al (DER # 11) effects on the behavioral parameters were observed at both doses tested (38 and 75 mg/kg/day) as assessed at 21 days, at which time statistically significant cholinesterase inhibition (approximately 20%) of the cerebral cortex was observed at both doses as well as statistically significant erythrocyte cholinesterase inhibition (also approximately 20%) at the 75 mg/kg/day dose level. Dr. Hartung says: "The spread between simple behavioral responses and cholinesterase inhibition argues against a need for further study." (p. 10) The converse of this is that further testing <u>would</u> be indicated if the said spread were small, or non existant, as is true in this case. He indicates his uncertainty as to what end points could be evaluated in the developmental neurotoxicity study, and would thus want assurances as to its interpretability before proceeding. This suggests, but does not say, he would support such testing were the test(s) meaningful.

Dr. Hartung questions the reliability of Russian neurophysiology, but without some reference to that literature with which to compare the work of Desi et al, it is difficult to appreciate any argument that the findings in Desi et al should not serve <u>at least</u> as a signal for definitive testing. It is documented in reliable sources that

EEG is responsive to cholinergic agents, see Ref. U, and thus if EEG changes are noted in studies at doses close to, or particularly below, those that inhibit brain cholinesterase as assayed, this would be an important end point of probable regulatory concern.

Dr. Decker is firm in his recommendation that: "..... additional neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations."(p. 3), by the best methods available. He also says, with regard to DER #11: "I agree with the Footnote on page 13 that the neurotoxicity and neurobehavioral testing should be greatly expanded in scope, in light of developments in these areas during the past decade. The DER should be put 'on hold' until these changes are made." (p. 3)

In my view, the responses of Drs. Hartung and Decker support a requirement for additional neurotoxicity testing that would be designed to reconcile the contrasting findings between the published and Guideline subchronic neurotoxicity studies in question. It is important to mention here as discussed elsewhere in this document that the publication by Kurtz (1977) (Ref. D) reveals a behavioral response to malathion within (actually below) the dose range that inhibited cholinesterase. The Guideline developmental neurotoxicity study, with some add-on testing, might be suitable to address the issue. While Dr. Dourson responds in the negative, his rationale does not incorporate or indicate consideration of the important issues being raised pertaining to neurotoxicity testing.

Question 2) **If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity? (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulatable end point (RfD) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not perfomed.)**

Dr. Dourson: Defers to EPA's experts.

Dr. Hartung: No answer.

Dr. Decker: Suggests having a neurotoxicologist provide criteria.

Comments: The consesus opinion is to defer the question to neurotoxicologists. These also must be external peer reviewers

VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

Supporting documentation: DER #s 1 - 3, 9, 10, 12 and 13; References: W, X, Y, Z and CC

Question 1) **Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?**

Dr. Dourson: Says maybe yes, but not so in the 2-year study now recommended by the Hazard ID Committee as the basis for the RfD.

Dr. Hartung: Says data are not presented in proper manner for his assessment.

Dr. Decker: Yes, more data needed to characterize the gender specific disparity

Comments: Dr. Dourson indicates while females may be more sensitive, they were not more sensitive than males in the 2-year rat study. It remains uncertain at this time as to just what the NOEL for erythrocyte cholinesterase inhibition may be in that study among females during the first 3 months of testing. Females were less sensitive on plasma cholinesterase inhibition in this particular study. It is unfortunate the data were not suitably displayed in order to gain the benefit of Dr. Hartung's opinion. Perhaps the possibility of follow-up with Dr. Hartung would remain in the event resoultion is not achieved without his comments. Dr. Decker considers the answer to be in the affirmative. In consideration of the responses to this question, and in view of the comments to the other questions in this section, a consesus exists that females are more sensitive.

Question 2) **What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?**

Dr. Dourson: Equivocal. Does not support the effort if the human study is not used.

Dr. Hartung: Supports evaluating the data base for the male/female ratio of sensitivity.

Dr. Decker: Says not his area of expertise.

Comments: The reviewers appear to recognize the importance of the task, but are not certain how to approach it.

Question 3) **Should additional testing in animal models be required to further quantitate the gender specific disparity?**

Dr. Dourson: No, to the extent the human study is not used.

Dr. Hartung: Yes

Dr. Decker: Yes

Comments: A consesus exists to pursue the task. If the human study is retained as the basis for the RfD, it appears the consesus would be elevated to one of unanimity.

VIII Cholinesterase Inhibition - Chronic Dog Study

Supporting documentation: DER #s 1 and 4; References: B (p. 4), H, I and AA

Question **Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g. subchronic feeding study, be required to characterize**

cholinesterase inhibition in the dog?

Dourson: No. However, his response appears to be predicated on use of an additional 3-fold uncertainty factor with the cholinesterase NOEL in the 2-year rat study.

Hartung: No.

Decker: Yes.

Comments: Dr. Dourson's response quite possibly would be different if the additional safety factor he recommends were not employed, particularly since he says elsewhere in his response: "I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (p. 4)

None of the reviewers offer any comments in response to issues raised in Ref. AA, certain of which are summarized as follows: 1) The subchronic feeding study was waived in the 1988 Malathion Registration Standard contingent upon the performance of a chronic dog study. In waiving the subchronic study, there is an enhanced burden for completion of an acceptable chronic study; 2) There are species-related biochemical similarities (absence of plasma carboxylesterase) to anticipate that the dog would respond similarly to man; 3) Cholinesterase methodology may be problematical in this 1987 study, and should be examined for conformity with the most current Agency standards; 4) The contrast between doses inhibiting cholinesterase in man and rat serves to indicate more definitive testing in a third species as FIFRA Guidelines intend; 5) The subchronic feeding study could possibly address the question of whether the manner of dosing is critical in the dog. The Hazard ID Committee should respond to these concerns

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A) Malathion - December 17, 1997 Report of the Hazard Identification Assessment Review Committee (Convened November 6, 1997.

B) December 17, 1997 Comments of B. Dementi on the December 17, 1997 Malathion Hazard Identification Assessment Review Committee Report.

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D) Kurtz, P. J. (1977) Dissociated Behavioral and Cholinesterase Decrements following Malathion Exposure. Toxicol. Appl. Pharmacol. 42, 589-594.

E) Pope, C. N. and Chakraborti, T. K. (1992) Dose-related Inhibition of Brain and Plasma Cholinesterase in Neonatal and Adult Rats Following Sublethal Organophosphate Exposures. Toxicol. 73, 35-43.

F) February 10, 1998 Letter of B. Dementi/Toxicologist to C. Swentzel/Chairman Hazard ID Committee.

G) A Two-Generation (Two Litters) Reproduction Study with AC 6,601 to Rats, June 28, 1990. MRID 41583401 (Selected Pages).

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I) November 10, 1997 Letter of B. Dementi/Toxicologist to C. Swentzel/Chairman Hazard ID Committee.

J) Two-Week (Range-Finding) Study of Aerosolized Malathion Administered byWhole-body Inhalation Exposure to the Albino Rat (July 20, 1993) (Selected Pages).

K) Organophosphate Action Update (January 1998) (Selected Pages). See "EPA Policies on Key Pesticide Policy Issues Clarified" (p. 2)

L) January 15, 1998 Letter of B. Dementi/Toxicologist to C. Swentzel/Chairman Hazard ID Committee.

M) March 28, 1995 Letter of B. Dementi, "Malathion Acute Neurotoxicity Study; MRID# 431467-01"

N) Toxicology Endpoint Selection Process - A Guidance Document Prepared by Jess Rowland, Health Effects Division, Office of Pesticide Programs. February 1997.

O) March 10, 1998 Letter of B. Dementi to Jess Rowland.

P) November 20, 1997 Letter of B. Dementi to C. Swentzel/Chairman Hazard ID Committee

Q) Desi, I. et al (1976) Toxicity of Malathion to Mammals, Aquatic Organisms and Tissue Culture Cells. Arch. Environ. Contam. Toxicol., 3, 410-425.

R) November 25, 1997 Letter of B. Dementi to C. Swentzel/Chairman Hazard ID Committee

S) May 4, 1995 Letter of R. MacPhail to J. Doherty and B. Dementi, Health Effects Division.

T) May 11, 1995 Letter of Brian D to Karen

U) Goodman and Gilman's The Pharmacological Basis of Therapeutics (1980) Selected Pages.

V) A Range-Finding Teratology Study with AC 6,601 in Rabbits (MRID 470088-012), Selected Pages Including Tables 3: Summary of Doe Body Weight During Gestation and Table 4: Summary of Doe Body Weight Gain During Gestation.

W) April 29, 1997 Letter of B. Dementi to E. Budd.

X) August 10, 1992 Letter of D. Allemang to L. Schnaubelt; October 8, 1992 Memorandum of B. Dementi to J. Edwards.

Y) January 22, 1993 Memorandum of B. Dementi to J. Edwards.

Z) Malaoxon Chronic Toxicity/Carcinogenicity Study: Selected Pages From DER #2.

AA) March 20, 1998 Letter of B. Dementi to C. Swentzel.

BB) Dementi, B. (1997) Cholinesterase Literature Review and Comment. Part A

CC) March 16, 1998 Letter of B. Dementi to J. Rowland.

MEMORANDUM April 8, 1998

To: Henry Spencer, Ph.D.
Manager, External Peer Review
Science Analysis Branch
Health Effects Division

From: Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I
Health Effects Division

Attached you will find questions (I-VIII), plus supporting reference documents, I am submitting to accompany the Hazard ID Committee report that will be going out for external peer review.

ATTACHMENT 13: Letter from B. Dementi - July 29, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

July 29, 1998

Re: Malathion External Peer Reviews, Follow-up Questions

This is an addendum to my memorandum to you of July 27, 1998.

Please find appended copies of letters from Dr. Michael Dourson (July 17, 1998) and Dr. Walter Decker (July 21, 1998) in which these external reviewers respond to additional questions posed by me after receiving their initial evaluations.

In Dr. Dourson's response to my first question, he expresses concern regarding the reliability of reported decreased pup weights during days 7 and 14 of lactation, which he says was due to chance, but concludes the LOAEL/NOAEL = 5000/1700 ppm for pup body weight changes based on findings at day 21 of lactation. This is in agreement with pup LOEL/NOEL identified in the DER. It should be noted that the pup weight decrements in question at days 7, 14 and 21 were all statistically significant findings. However, Dr. Dourson did not address clearly my real question, which is whether the evidence of greater sensitivity of pups versus adults in this study can be reliably discounted using the arguments put forward by the Hazard ID Committee without a showing of malathion in the milk (and how much is there) and without any data to indicate how much solid food pups may have actually consumed during lactation.

In Dr. Dourson's response to my second question, he asks whether the effect on avoidance behavior was statistically significant. Kurtz (1977) says that it was statistically significant, $p < 0.02$. (p. 590) Further, the publication says: "... significant behavioral decrements were found at dosages producing only negligible changes in ChE activity: The median avoidance latency of the group tested 1 hour after injection with 50 mg/kg was 12.2 sec compared to 0.6 sec in the control group, but ChE activity of this group was greater than 90% of control values for all three ChE measures." (p. 591) Consideration of this and other information in the Hazard ID Committee reference materials, and Dr. Dourson's comments in his item 3 of question 2, would indicate some recognition on his part of the need for conducting the developmental neurotoxicity study on malathion. He says at the very least, he would ask that the i.p. study be repeated with more animals and more behavioral tests. Clearly the concern is real.

In Dr. Decker's response to the question posed by me regarding the inhalation study, he says no to the 1/3 LOEL, and advocates an interim 1/10 LOEL for the inhalation study, while assuming, " of course, that further testing will be forthcoming to determine a NOEL, ..."

Brian Dementi
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 14: Letter from B. Dementi - August 3, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

August 3, 1998

In preparation for the August 18, 1998 Hazard ID Committee meeting on malathion, I have a few additional comments regarding the interpretation of the malathion two-generation reproduction study in the Sprague-Dawley rat (MRID 41583401) (DER #5)

The December 17, 1997 Hazard ID Committee report covering the November 6, 1997 meeting to consider malathion says: "For parental systemic toxicity, the NOEL was 5000 ppm (394/451 mg/kg/day in M/F) and the LOEL was 7500 ppm (612/703 mg/kg/day in M/F) based on decreased P generation body weights during gestation and lactation and decreased F1 pre-mating body weight." (p. 15) The problems I have with this, as gleaned from the DER of the study, are explained as follows: 1) Parental (dam) body weight was not affected at any dose level during either of the two F1 lactation periods, i.e. for litters F2A and F2B, as recorded in Table 3 (p. 11) of DER #5, copy appended; 2) Parental (dam) body weights were significantly less in the 7500 ppm dose group for both of the F0 lactation periods, i.e. for litters F1A and F1B as recorded in the same Table 3. However, in the case of both of these F0 lactation periods the effects were most remarkable on lactation day 0, a day which follows immediately on the heels of delivery and more properly should be viewed as a manifestation of effects incurred during gestation and delivery. The meaningful period for assessing dam body weight effects of/during *lactation* rests with what happens *after* Day 0, i.e. on days 7, 14 and 21 in this case. As I examine this data during both of the F0 lactation periods, I observe considerable recovery of dam body weights by day 7, and that *changes* in body weight assessed across the 21 day period (e.g. days 7-14, 7-21, 14-21) in all dose groups appear to be essentially unaffected at any dose level (I say this without the benefit of statistical analysis, which I recommend be done). To the extent that the body weights in the 7500 ppm dose groups remain less than the control post day 0 is arguably a carry over of the Day 0 deficit, since there is little or no evidence at any subsequent time point of further erosion of body weight. Again, during both F0 lactation periods, dams show evidence of recovery post Day 0. My view is that dams in the 7500 ppm dose group were affected during pregnancy insofar as indicated by body weight deficits on lactation Day 0, but no conclusive evidence exists to show *during* lactation that dams were affected at any dose level in any of the four lactation periods under study; 3) Decreases in dam body weight during *gestation* in my view cannot be interpreted to be uniquely parental/dam effects; 4) During the pre-mating period there were no effects on F0 male or female body weights, Table 1 (p. 9) of DER #5, copy appended. However, there were statistically significant body weight decreases in both F1 males and females at the 7500 ppm dose during the pre-mating period. While this may suggest a parental effect at 7500 ppm, it must be recognized that F1 animals, unlike F0 animals, were exposed to malathion in utero and, hence, effects cannot be divorced from a possible fetal/developmental etiology. So to the extent that the reproduction study is employed to differentiate possible differences in sensitivity between young/developing individuals and adults, as required under FQPA, effects on F1 parental animals are to be of questionable usefulness. In the case at hand, the fact that body weight effects were observed in the F1 animals at 7500 ppm during premating, but not in F0 males or females during premating is supportive of a possible adverse effect of the test material on F1 animals during development, manifested as an enhanced adult sensitivity.

The bottom line is that there are no unencumbered body weight data in this study that shows an adverse effect of malathion at any dose level on adult animals apart from possible effects on the developing animal. Evidence which has been cited in support of an effect at 7500 ppm is indefensible. Effects on body weight during pre-mating were only on

F1 animals, which were exposed during development. During lactation, there were either no effects (F1 lactation periods), or effects seen at Day 0 (F0 lactation periods) tended to recover and/or got no worse *during* lactation and, hence, cannot be said to represent effects peculiarly on dams divorced from possible consequences of effects on the developing individuals. Similarly, dam body weight changes during gestation cannot be used to deminstrate a peculiar effect on adult animals.

So, as I have said previously in my letters to Jess Rowland (December 17, 1997; Ref. B) and to you (February 10, 1998; Ref. F), body weight changes and other parameters evaluated in reproduction and developmental toxicity studies do not provide adequate information to identify possible greater sensitivity of young/developing animals versus adults. But even to the extent that body weight changes in adults and offspring evident in the two-generation reproduction study on malathion have been used for this purpose, closer examination of the DER does not reveal any indisputable or *reliable* evidence of an effect of malathion on body weight changes in adults at any dose level, either during *gestation*, *lactation* or *pre-mating* periods as claimed in the Hazard ID report. Effects on offspring occurred at 5000 and 7500 ppm, and possibly at all doses of the F1A litter during lactation in terms of body weight deficits. The study thus supports a greater sensitivity of the developing organism.

Our experts on reproduction toxicology should be invited to examine the study closely and comment on the views I have expressed for the benefit of the Hazard ID Committee.

Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 15: Letter from B. Dementi - August 10, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

August 10, 1998

As explained in my letter to you of August 3, 1998, closer examination of the DER (#5) for the 2-Generation Reproduction Study for malathion does not reveal any indisputable or *reliable* evidence of an adverse effect of malathion on body weight changes in parental animals at any dose level, either during *gestation*, *lactation* or *pre-mating* periods as claimed in the December 17, 1997 Hazard ID report. This would mean that for the said study the parental toxicity NOEL $\geq$ 7500 ppm (HDT), while the developmental NOEL/LOEL = 1700/5000 ppm.

Again, in preparing for the August 18, 1998 meeting, I have now examined the Study Report of the 2-Generation Reproduction Study for further details and must advise the Committee that the **Study Report** (MRID 41583401), in contrast to the DER (#5), concluded there was no adverse effect on parental animals: "Thus, in this two generation reproduction study in rats involving continuous treatment with AC 6,601 in the diet, the parental no-observed - advers-effect level (NOAEL) was 7500 ppm and the NOAEL for developmental toxicity was 1700 ppm." (p. 6) The principal reason for this discrepancy between the DER and the Study Report rests with the reporting of parental body weight data. The DER reported only mean body weights of parental animals at critical time points, such as pre-mating, while the Study Report provided data showing changes in body weight as well. Thus while body weight in a 7500 ppm parental group may have been less than the control, body weight changes during the period in question were unaltered.

For example, in the case of pre-mating parental body weight data, for the F0 parental animals there were no treatment related effects of dosing on body weight. However in the F1 parental animals, while mean body weight in the 7500 ppm group was less than that of controls, there was no effect during this period on body weight *gain*, a finding neither discussed nor noted in the DER (#5). The Study Report says the following with respect to the F1 parental animals: "In Group V (7500 ppm), mean weekly weight data for males and females during the pre-mating treatment period were lower than control and these differences, throughout this interval, were statistically significant. Mean weight gains, however, over the entire 10 week pre-mating period for these Group V animals (both sexes) were comparable to control data. Thus, while Group V animals initiated the pre-mating treatment period smaller than control animals, and ended the period smaller, the weight gain experienced by these two groups over the entire period was considered comparable. Thus, no adverse effect of treatment up to a dietary level of 7500 ppm was evident from weight gain data during the pre-mating treatment periods for either parental generations (P1, F1)." (p. 28 of the Study Report). In my view this assessment in the study report is supported by the data presented in that report, is entirely correct and indicates a need for revisions to the DER (#5) to present a more satisfactory interpretation of the findings where the relative sensitivities of adult versus young/developing animals is concerned. This need is more critical now that the reproduction study is being relied upon to make such destinctions as required under FQPA.

Similarly, the study report provides data showing that mean weekly body weights during the mating and post-mating periods for F0 male animals to produce the F1a and F1b litters, were comparable between the control and treated groups. By contrast, in the case of F1 males (which unlike F0 animals were exposed in utero), weekly body weights

during the mating and post-mating periods to produce the F2a and F2b litters were statistically significantly lower in the 7500 ppm group than the control, but are only consistent with the lower weights seen in this group during the pre-mating period. The study report says : "Thus, no adverse effect of treatment up to a dietary level of 7500 ppm was indicated from weight gain data for males during the mating and post-mating intervals for either the P1 or F1 generations." (p. 33 of the study report) Again, I find this conclusion entirely supportable by the data in the Study Report, which is simply not conveyed forward in the DER (#5).

The bottom line to all this is that the data in the Study Report do not support a conclusion that parental animals were affected at any dietary level of malathion tested as gleaned from body weight data. The DER (#5) should be revised to reflect these findings and is a matter that should be commented on by the Committee.

This further supports what I indicated earlier, namely, to justify removal of the FQPA imposed 10X factor, there is a larger gap between the developmental NOEL/LOEL (1700/5000 ppm) and the parental NOEL ($\geq$ 7500 ppm) to be explained away by the Hazard ID Committee than was considered to be the case at the November 6, 1997 meeting.

Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 16: Letter from B. Dementi - August 17, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

August 17, 1998

In response to your memorandum of August 14 concerning the format for the August 18 meeting of the HIARC (copy appended), I am concerned that the discussion may be restricted to the eight topics generated by me that were submitted for external review. I say this because Dr. Hank Spencer, the external peer review coordinator, introduced certain preliminary questions that were also responded to by the three scientists in question, Drs. Michael Dourson, Rolf Hartung and Walter Decker, that require assessment by the Committee. These questions pertained to the acceptability of the various malathion DERs, whether critical effects were chosen in the various studies and whether the data base is complete. One of my principal concerns as expressed in my December 17, 1997 comments to Jess Rowland, Ref B in the background package, was that of whether there are data gaps in the malathion data base. As the Committee is aware, removal of the 10X safety factor under FQPA for the protection of infants and children requires a complete data base. In consideration of the fact that the external reviewers had much to say regarding the adequacy of the data base and data gaps, I had planned to mention this to the Committee.

Pertaining to the acceptability of the malathion data base, the following are noteworthy statement rendered by the external reviewers.

Dr. Dourson says "The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurment of RBC cholinesterase inhibition in the 2-generation study is a data gap....." (p. 3) "The specific question to be addressed with these data are whether or not the NOEL of the likely critical effect after 1 day exposure is determinable. The available data in this review, including the developmental studies in rabbits, do not allow this question to be answered." (p. 3) "No, the data on which to make this determination are absent." (p. 5) "However, I believe that the rat NOEL should be further divided by a 3-fold uncertainty factor to account for deficiencies in the data base...." (p. 8) "However, it does not test females, so the NOEL/LOEL range could be lower." (p. 8) His responses to both questions IV and V calling for additional information indicate his recognition of the existence of additional data gaps. A most significant statement made by Dr. Dourson reads as follows: *"I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (p. 3)*

Dr. Hartung, beyond saying that a toxicology data base is never complete (p. 4), does not particularly address the question specifically for malathion. He does say the following: "The available data is inconclusive whether a single dose, administered during a day of maximum sensitivity would be able to elicit the observed response, or whether cumulative dosing is required." (p. 5) "This requires an analysis of the detailed cholinesterase methodology." (p. 7)

Dr. Decker: "The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups. The tumors should be further histologically defined." (p. 2) Along these same lines, he indicates that these findings "...demand further testing in a larger group of animals in all dosage groups." (p. 4) "The finding that the increased numbers of hepatocellular tumors observed in the male mice at 100 ppm as compared to the lower numbers of such tumors observed at 800 ppm is not interpretable, in my opinion. Rather, this part of the study should be repeated. The rest

of the study seems to follow the Guidelines well, and appears to be scientifically valid." (p. 2) "I agree with the EXECUTIVE SUMMARY that this study is not acceptable and does not satisfy Guideline 83-1 for a chronic toxicity study in dogs because NOELs were not established for cholinesterase activity inhibition for plasma and erythrocytes in either sex." (p. 2) "Lacking an answer to this question, I would recommend that this DER be changed from CORE MINIMUM to UNACCEPTABLE for the section of the report on eye histopathology." (p. 3) "Although this study appears to satisfy the requirement of Guideline 82-7 for subchronic toxicity determinations, it was correctly pointed out in the Study Classification section that other published data indicate possible evidence of neurotoxicity on parameters not assessed in the 82-7 Guidelines. I recommend a thorough literature search on theses and that the results be used to construct additional specific neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations." (p. 3) "I agree with the Footnote on page 13 that the neurotoxicity and neurobehavioral testing g should be greatly expanded in scope, in light of development in these areas during the past decade. The DER should be put "on hold" until these changes are made." (p. 3) "This study seems to be generally acceptable, but does not satisfy all requirements of Guideline 82-4, since no NOEL was established for plasma and RBC cholinesterase inhibition in female animals or for microscopic lesions of the nasal cavity of the larynx in both sexes." (p. 3) "I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in data gaps." (p. 4)

These various views rendered by the external scientists serve to underscore my expressed opinion that it cannot be claimed, as was done in the December 17, 1997 report of the HIARC that "The toxicology data base is complete and there are no data gaps." (p. 18) This latter statement is offered as satisfying one of the requirements under FQPA that must be met before the 10X safety factor, imposed for the protection of infants and children, can be removed. I doubt very seriously that Congress intended that anything other than fully acceptable studies, with no data gaps of the nature identified by the external reviewers, could be used to satisfy this very important criterion for removing the said 10X factor. I must express additional concerns I have regarding the procedures to be followed for the August 18 meeting as expressed in your August 14 memorandum. As you know I have many contrary views respecting those of the HIARC over the adequacy of the malathion data base, and I must insist upon the freedom to express my views. There is much food for discussion resident both in the several memoranda I have addressed to the Committee since the November 6 meeting, and in the comments of the external reviewers. I consider it unfortunate that so many issues are contemplated for this one meeting, and am concerned therefore that each issue may not be accorded the time needed in the press to cover all the issues in one meeting. I am also concerned over the assignment of certain members of the HIARC to the various questions, as this may have a negative effect on the extent to which other members of the Committee evaluate all of the issues, i.e. too much reliance of the Committee as a whole may be placed on the opinions rendered by the one principal reviewer in each case. Of course, I would hope and trust this would not be the case, but I would also hope that each member of the Committee would be invited to express his/her views on any and all questions after having thoroughly studied the full data base. As I have found it to be true at various HED Committee meetings, issues/questions often arise that have no adequate response at the time, and certainly in my case, I sometimes don't provide the quality of answer I might given more time to reflect on various issues, as these often are complex. Hence, there simply must be opportunity for follow-up after these meetings, before final reports go out. Nothing, including the press of time, should preclude gathering and expressing the facts, no matter how far a hearing has advanced, where public health matters are of concerned.

Brian Dementi, Ph.D., DABT
Toxicologist, Toxicology Branch I/HED

ATTACHMENT 17: Letter from B. Dementi - September 24, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

September 24, 1998

Please find appended a copy of the following journal publication from the open literature: Mendoza, C.E. (1976) Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats. Toxiol. Appl. Pharmacol., 35, 229-238.

This is being offered for possible inclusion under section III (2) (iii) "Information from the open literature" (p. 16) in the December 17, 1997 "Report of the Hazard Identification Assessment Review Committee" on malathion, as relevant to the Determination of Sensitivity for FQPA Considerations

Perhaps the person who assimilated the literature review section of your report would find this publication both interesting and relevant, and wish to incorporate it in the review. I have read the article, which leads me to conclude that it provides information indicating younger animals to be more sensitive to malathion, but have not had time to review it.

Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 18: Letter from B. Dementi - November 5, 1998

Clark Swentzel, Chairman November 5, 1998
Hazard Identification Assessment Review Committee
Health Effects Division

Comments I would offer to the October 27 draft report of the August 1998 Malathion Hazard Identification Assessment Review Committee are offered below.

I SPECIFIC COMMENTS

P. 1, paragraph 2: The August HIARC meeting occurred on August 18, 20 *and 27*, 1998.

P. 1, paragraphs 2 and 3: You say HIARC evaluated the comments and responses provided by the external peer review. Actually the statement should include affirmation of the fact the committee was also considering the comments presented in some fourteen memoranda on toxicology issues submitted by Dr. Dementi, many of which served as the impetus for the external peer review and in fact were addressed by the peer review members. You cannot divorce these memoranda from the deliberative process in presenting an historically correct record. You should also say at this point that these memoranda by Dr. Dementi constitute part of the record of the deliberative process.

P. 2: Karle Baetcke was not in attendance at any of the meetings I attended on August 18, 20 or 27.

P. 3, paragraph 2: You say "Following that meeting, the Agency conducted an external peer review of a number of issues related to hazard identification for malathion." From whence did those issues arise? For the benefit and enhancement of the understanding of your audience, this statement should be more forthcoming in laying down the historical record and rationale for that external review. Accordingly, your statement might be rephrased thusly: *"Following that meeting, the Agency pursued the external peer review mechanism to address the number of issues raised by HED's malathion toxicologist following the November 6, 1997 HIARC meeting."*

P. 3, paragraph 2, 3d line: *experts*

P. 3, paragraph 3: August 18, 20 *and 27;* responses; "..... of the external peer review panel *and Dr. Dementi.*"

P. 3, paragraph 4: *Michael; Rolf;* in addition to the eight major topics, you should acknowledge the preliminary questions concerning the general acceptability/completeness of the data base posed by Dr. Hank Spencer, HED's external peer review coordinator. You say the Panel received all pertinent reference materials. However, you should go a little further in informing your audience as to what these materials were, namely study DER's, one-liners and Dr. Dementi's memoranda and set of questions.

P. 4, paragraph 2: Delete *Do the rabbit*

P. 4, paragraph 4: *range-finding; main rabbit; considered*

P. 4, paragraph 4: Under HIARC's justification for the acute oral (one-day) end point, see my comments on page 6

(paragraph 4) of this document analogizing to oral exposure HIARC's former assumption that no effects would have been expected early into inhalation exposure.

P. 4, paragraph 5: *the acute RfD; These decreases;*

P. 5, paragraph 3: *error in equation, 50 mg/kg/day/100 (UF) =* ***0.50*** *mg/kg/day. It is noteworthy that this dose is 21.7 (12.5)-fold that of the RfD of 0.023 (0.04) mg/kg/day, depending upon whether the human or rat study serves as basis for the RfD.*

P. 5, paragraph 4, last line: ....*study* sufficiently....

P. 5, paragraphs 5 and 6: Your report does not provide the Panel's response or HIARC's conclusion relevant to the all important question 3) c) namely, " Is the data available in the developmental study sufficiently reliable to discount the 10X safety factor required under FQPA?" Of course, the Panel's opinion was unanimous that it is not.

P. 6, paragraph 2, last line: ".....evidence (of parental toxicity) is not strong." More needs to be said here by way of qualifying this remarkable statement. If the evidence is "not (as underscored) strong", how can it satisfy as *reliable* data for the protection of infants and children as specifically required under FQPA? You must explain that obvious anomaly. In my view as expressed at the HIARC meeting, the study does not satisfy as showing a parental effect at any dose level which means there are pup body weight effects at two doses in the absence of parental toxicity, thus establishing greater sensitivity of the young and developing individual, the specific concern of FQPA. It was my understanding at the HIARC meeting on August 20, that Dr. Dapson was going to provide a written supplemental review of the data in this study after it was pointed out that the author of the MRID study report had concluded there were no parental effects at any dose level and that this was based on body weight *gain* data presented in the study report that had *not* been incorporated into the DER. Furthermore, the study author had concluded that offspring were adversely affected at both the top and penultimate dose levels. I must ask the question as to whether any re-review of this particular aspect of the study has been undertaken by the committee that drives your qualifying remark about the finding *not* being strong. Any rationale supporting this new claim must be presented for all to see.

P. 6, paragraph 5: Your statement in bold print is troubling to me. The statement as a whole does not bespeak of the kind of certainty that I believe was the intent of Congress in calling for *reliable* data. Furthermore, I do not recall this statement as consistent with the tenor of the discussion held on August 20, but rather strikes me as some sort of new rationalization developed since that meeting. Have there possibly been other meetings of the HIARC held since August 27? You also say in this paragraph that "The presence of the chemical in the milk is a generic assumption .....(unless we have data to show otherwise)...." Your record must show that at the August 18 meeting, when this issue was first visited, Dr. Protzel left the meeting to retrieve the residue chemistry metabolism study. That study, performed in the goat, revealed only two non-cholinesterase inhibiting metabolites of malathion, i.e. malathion was not present in the milk. Furthermore, I subsequently spoke with Mr. Bill Smith, malathion team chemist, who confirmed via the spoken word, that malathion is not a residue in milk. So the condition for your generic assumption to apply has not been met by the Agency's actual testing procedures as required under Residue Chemistry to set tolerances. This information should be recorded in your report, particularly the effort on Dr. Protzel's part as that actually took place at the meeting. What's more, the committee was reminded on August 20 while the milk issue was still under discussion that Dr. Protzel had obtained the data at the previous meeting on August 18 and it was negative for malathion. Furthermore, the committee needs to revise its conclusion as to the use of the milk arguement in discounting relative sensitivities of young and developing individuals versus adults in the reproduction study. I wish to reaffirm here the view I expressed at the August meeting that the reproduction study reveals a greater sensitivity of the

young and developing individual, and that arguements to the contrary simply "don't hold water", paricularly so in view of Congess' qualifier in FQPA regarding the need for *reliable* data.

P. 6, last paragraph: I don't affirm this line of reasoning as having taken place at the August meeting. However, if it was, the question needs to be referred back to the FQPA Safety Committee. I am certain that committee would want the correct data in place for the rendering of its opinions, and that committee should also now be privileged to have the benefit of the external reviewers' opinions in addressing the issues. However, contrary to this you do say on p. 1 that the HIARC committee "....addressed the sensitivity of infants and children from exposure to malathion as required by the Food Quality Protection Act (FQPA) of 1996." So to the extent HIARC did in fact perform this assessment as claimed, you should not disown this responsibility by placing it on the shoulders of the FQPA Safety Committee as you're doing on p. 6. **All things said, the Agency's obligation is to the protection of the public health, and the scientific facts are what count in setting end points and applying safety factors, regardless of which committee assumes the burden of rendering the judgement.**

P. 7, paragraph 3: The committee's conclusion does not address the question of FQPA's insistance upon *reliable* data. Does this study meet the test of providing *reliable* data for the protection of infants and children under FQPA. The expert panel has said no. How can the committee justify to the public a decision differing from this in discounting the 10x factor required by Congress?

P. 7, between paragraphs 4 and 5: "Panel's Response" to this very critical question seems to have fallen through the cracks. In response to this question, you Panel's Response should record that Dr. Dourson suggested a 3X safety factor as opposed to 10X, while acknowledging 10X may still be useful as a management tool. Drs. Hartung and Decker say no, though Dr. Hartung insists offspring must be shown to be less sensitive. Also the external reviewers were not aware of the more recent concern that the DER for this study did not address the study author's observations that body weight *gain* data, not shown in the DER, do not support a conclusion that adult animals were affected at the highest dose. Nor were the external reviewers aware that malathion has been shown not to be present in milk, thus removing one principal reason HIARC employed to discount differences in sensitivity between offspring and adult animals in the said reproduction study.

P. 7, paragraph 5: Among the basic Guideline studies, only the developmental toxicity and reproduction studies assess relative sensitivities of young and adult animals. You need to make that clear here. To the extent the reproduction study fulfills this role, the external reviewers have said the study does not provide such reliable data. That taken in concert with data showing greater sensitivity of young animals in this study, as I believe it does, leads me to doubt very seriously the public would take much comfort in the generic issue arguement being waged here to discount the absence of satisfactory data to make the needed destinctions between young and adult animals.

P. 8, paragraph 5, line 5: In order to adequately convey to your audience the assessment of Dr. Dourson when using his quote, firstly the quote should read as follows: "..... *principally because the critical* ***effect*** *was not monitored in the two-generation reproduction study in a potentially sensitive subgroup (i.e. young rats).*" Secondly, Dr. Dourson is speaking here of but *one* critical effect, namely cholinesterase inhibition. My claim in identifying that particular effect as cholinesterase inhibition is supported by the following statement of Dr. Dourson: "The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurement of RBC cholinesterase inhibition in the 2 generation study is a data gap that can best be addressed through the use of a 3-fold uncertainty factor when determining the RfD." (page 2 of Dr. Dourson's June 3, 1998 submission). It is important that you make your audience aware of the identity of the effect (cholinesterase inhibition) because it is the basis of the RfD, and that Dr. Dourson considers it a data gap. The full weight of his testimony should be conveyed here. **Also,**

since you have quoted from Dr. Dourson, a balanced approach would necessitate quoting the other external reviewers. These quotes are not long. *Dr. Hartung*: **"No. The human is the correct species of concern**. **Substituting a rodent introduces many more uncertainties than those produced by minor deficits in the analysis of chemical purity or concern about statistical precision."** (p. 7 of his 6/3/98 comments); and **"Look at what you are doing! Here you are willing to accept a study for which you are also willing to mess around with another factor of 10X, just because the statistical data are neater. In the process you are willing to discount human data, even though it is extremely unlikely that the equivalent statistical uncertainties for the human will reach anywhere close to 10X."** (p. 8 of his 6/3/98 comments). Note he addresses the purity question, and I advised the committee that the human study in question, Moeller and Rider, while not stating the purity, did claim it to be American Cyanamid malathion, the purity of which was known in the industry at the time. Furthermore, at the committee meeting there was an extensive discussion of the fact that the rat may be a poor surrogate for man, based upon differences in carboxylesterase profile in rat versus man. The committee even concluded on August 18 to impose the additional 3-fold uncertainty factor, which the committee reversed on August 20 because the issue may relate to other pesticides where it has not been addressed. *This aspect of the deliberations finds no entry in these draft minutes. Dr. Decker*: **"Additional testing should be required in the male <u>and</u> female rat before any thought is given to replacing the human data relied on to establish a RfD."** (p. 5 of his 6/11/98 comments)

You should say here something such as: *In summary, two external reviewers were firm in recommending against switching to the rat study, while the third member favored the rat study, contingent upon imposition of an additional 3-fold uncertainty factor. The committee is ignorant as to the latter's views regarding the use of the rat versus the human study in the absence of an imposed additional uncertainty factor.*

P. 8, paragraph 6: For the full understanding of your audience, remembering the importance of *transparency* in our products, you should say something at this point to the effect that: *In this HIARC decision, the recommendations of **all** external reviewers were discounted.*

P. 9, paragraph 3: The Panel's Response as described is incorrect in light of the following: 1) *Dr. Dourson* advocated 10X as opposed to 3X. 2) *Dr. Decker*, in his follow-up response of 7/21/98 says: **"Based on my experience (43 years in the field of toxicology), Reference N (TES Process), and the letter from Dr. Dementi (July 9, 1998), I doubt that the 1/3 LOEL is adequate to account for the absence of a NOEL. At the present time it would seem prudent to use 1/10 LOEL." I assume, of course, that further testing will be forthcoming to determine a NOEL, at which time this safety factor should be reexamined."** 3) *Dr. Hartung* says: **"This fine-tuning is unwarranted because of major species differences in exposure scenarios."** This should be interpreted to mean that fine-tuning, 3X or 10X, in his view cannot address the inadequacies. It cannot be taken to mean he opposes increasing the factor from 3X to 10X. Indeed, given his expressed views, proper testing is indicated, but lacking that and until proper data is in place, the implications of his words *convey to me* that he would consider 10X as preferred for public health protection, although he does not actually say that. The bottom line is that two reviewers, a consensus, supports the imposition of a 10X safety factor, while the views of the third should be suitably qualified in your report and cannot be simply cited as "one member recommended against the use of an additional UF", left to be interpreted to mean Dr. Hartung sees no need to increase the uncertainty factor because the study is adequate as it stands. Again, *transparency* of your presentation is the issue here regarding Dr. Hartung's comments.

P. 9, paragraph 4: This HIARC conclusion is incompatible with my notes and recollections of events that transpired at the August 27 HIARC meeting, a meeting which, incidentally, is not even acknowledged in these minutes as having

occurred. *This is peculiar and of great concern to me.* At that HIARC meeting, in my witness the committee's designated "expert" recommended and the committee adopted raising the UF from 3 to 10. There is no mistake in this. Does this conclusion possibly reflect deliberations of the committee that took place at another time in my absence? If so, the minutes of any such meeting, including the date, who was present, etc. should also be a matter of record and noted here, for the sake of historical accuracy, if nothing else. If another meeting after August 27 did *not* occur, are these draft minutes to be viewed as perhaps anticipatory of what is yet to be presented, suggesting selective prior knowledge, in which case they are not all minutes of past events and should require no response at this time, for how can one be expected to comment on an event he never witnessed or attest to events yet to occur.

According to my witness, the HIARC Conclusion should say, for example, *The HIARC concluded that the Margin of Exposure should be increased from 3X to 10X, for both Intermediate and Long-Term inhalation exposures.*

P. 9, paragraph 5: The rationale presented here relates to question 2. Since in actuality, you did not address question 1 in your conclusion, the rationale for your decision as presented in this paragraph 5 is irrelevant and immaterial insofar as it puportedly relates to question 1.

P. 9, paragraph 7: *members; suggest.*

P. 9, paragraph 8: HIARC Conclusion should record that the committee decided to invoke an MOE for short term acute risk assessment for the reason that the effects of concern were seen in a two-week study. However, the conclusion should also reflect the August 27 decision to invoke the same 10X factor for short term as for the intermediate and long term endpoints.

As an aside I should note here the committee was too quick back in November, 1997 to deny the need for an MOE, by assuming, in the absence of short term data, that effects would not occur in the short term. I should also note that Dr. Dourson's comments suggesting the inhalation data do not support cumulative effects for cholinesterase inhibition, leading him to say "..... that an extra uncertainty factor for potential cumulative effects is not needed." (p. 10 of his 5/29/98 comments) are not only germane to inhalation exposure MOEs, but to the question of the committee's acceptance of an acute, one-day RfD as high as 0.50 mg/kg/day (based on non-cholinesterase data) as contrasted with the longer term cholinesterase data derived RfD of 0.023 (or 0.04) mg/kg/day, a 21.7 (12.5)-fold difference, wherein again it is being assumed, in the absence of short-term oral data, that cholinesterase would not be as responsive over the course of 1-7 days as it is beyond this time frame. Protection of the public health demands more than assumptions in setting these important end points, whether they be inhalational or oral end points. Until short-term (1-7 day) oral cholinesterase data are available, one RfD for all time points should be employed.

P. 10, paragraph 3: In my witness, the HIARC Conclusion offered here is inconsistent with my understanding at to the committee's conclusions rendered August 27. According to my records, the committee imposed an uncertainty factor of 10 on all three end points. Furthermore, the committee decided to require another inhalation (nose-only) study in the rat. The requirement for this study was driven primarily by the nasal tissue effects, for which there was no NOEL in either study. I do not recall any discussion having taken place concerning comparisons of derived NOEL for histopathology versus the NOEL for plasma cholinesterase inhibition, nor any arguements as to usefulness of a nose-only study. **In my view, one cannot predict what the nose-only study will show regarding effects on the nasal tissues, which needs to be addressed**. Until such work is done, the added 10 UF is called for, as disclosed in the committee "expert's" August 27, 1998 submittal to the committee, and as supported by HED's February 1997 "Toxicology Endpoint Selection Process".

More specific to your comment, the derived NOEL of 0.003 mg/L shown in your document should be given as 0.001 mg/kg according to the committee's decision to employ the 10X uncertainty factor. I Am not certain of the point you attempt to make in contrasting a derived NOEL for nasal effects versus a NOEL for cholinesterase inhibition. Why not versus a derived NOEL for cholinesterase inhibition of 0.0045 mg/L? The contrast would then not seem so remarkable. Yet, the DER claims there was no NOEL for cholinesterase inhibition where concentrations tested were 0, 0.1, 0.45 and 2.01 mg/L. The Agency employs safety factors of 10X from animal to man and another 10X for variations in human sensitivity, so the bulk of the contrast you cite (1500 fold) rests with these legitimate factors universally applied for the protection of the public health.

P. 10, paragraph 4: I do not recall this conclusion as having been reached during any of the three meetings of the HIARC in late August. Am I to conclude from this that these various conclusions with respect to the inhalation study were drawn at a meeting I was not privileged to attend? If so, the date, participants, etc. should be incorporated in this record. In any case you say: "If another study is conducted, it would have to be 'nose-only' exposure in which case the NOEL/LOEL will be higher." Higher than what? There is no NOEL. Further, if you mean the atmospheric concentration eliciting *nasal tissue* effects, it is necessary that you present reference material showing that *nasal tissue* effects as opposed to non-respiratory tissue effects are differentially affected in the two kinds of studies. However, even that would be inadequate since each test material potentially has its unique effects on nasal tissues, and whether there is a systemic component is knowable only on a compound by compound basis. Since this has not been done for malathion, it cannot be presumed to fall one way or the other in the absence of testing, particularly since the effect in the existing study is said to be severe. Logically, and in being consistent with your obligations to protect the public health, further testing should replace presumptive rationalization. It is my understanding that excepting *local* respiratory system effects (as opposed to *systemic*) effects, the whole body assay is conservative and when negative is acceptable. However, when positive, a repeat nose-only study may yield less sever *nasal* effects only if oral ingestion contributes to expression of the effect. So if that is the case, testing by the latter procedure *may*, indeed, get one "off the hook". Nonetheless, when the effect precluding assignment of a NOEL is a respiratory system effect, additional testing is necessary at lower concentrations to identify a NOEL. Until that time, because the effects on nasal tissues are described as sever and occurring in essentially all animals at the LOEL, a 10X as opposed to 3X factor must be imposed. I should remind you this was the recommendation of a consensus of the external reviewers and your committee's designated "expert" at the Augusty 27 meeting. Nothing has changeed since then, at least from my perspective. **Your statement represents a presumption that nasal tissues would be differentially affected in the two kinds of inhalation studies that negates proper end point selection in the face of a glaringly positive finding with no NOEL. That cannot be accepted in lieu of actual data**. **The purpose of another study would be to identify the NOEL for nasal tissue effects, as this has not been identified in any existing study**. Also, don't forget there was no NOEL for the effects in question after only 2-weeks of testing in the range-finding study, suggesting effects on nasal tissues are of early onset, which should be a weighing factor in your assessment for the need of additional subchronic testing to identify NOELs.

P. 11, paragraph 2: You embolden the last sentence as if to cast aspersions on the appropriatness of the question of whether the chronic toxicity/carcinogenicity study weighs at all in the decision to retain or discount the FQPA imposed 10X safety factor. Well obviously the study makes no distinction between susceptability of young and old animals. However, I am often troubled by statements such as that on your p. 7, paragraph 5, where it is said: "At present the determination of susceptability is made not based on the results of one study (where in fact one *appropriate* study that is positive will do) but rather on a *weight-of-evidence* (emphasis added) basis that includes acute and subchronic neurotoxicity studies, the prental developmental toxicity studies in rats and rabbits, the 2-generation reproduction toxicity study in rats as well as *the toxicity profile of the chemical* (emphasis added). I put this question forward to make it *transparent* to observers that this major study (combined chronic toxicity/carcinogenicity) does not contribute

anything magical to the claim of the weight-of-evidence toward justifying removal of the 10X safety factor for the protection of infants and children. In my view illegitimate mileage is often reaped under the claim "weight-of-evidence" when in fact the well may be rather dry. Where the FQPA 10X factor is concerned, if young and developing individuals are shown to be more sensitive compared to adults in either or both developmental or reproduction studies, the factor remains. In fact, your embolden statement says as much here.

P. 11, paragraph 5: HIARC conclusion notes an *ad hoc* subgroup report of November 13, 1997. There is nothing new here that might serve to overide the recommendations of the external reviewers. In fact it was in part due to my differences of opinion with respect to the conclusions of the *ad hoc* committee that prompted the Agency to invite external toxicologists to vote on these differences of opinion. The external reviewers, with the *ad hoc* committee report before them, in addition to my assessments and the study DERs, confirmed the position advocated in my reviews. Also, I find it regrettable that the HIARC does not even acknowledge, let alone address, additional comments that I, in good faith, submitted to the committee dated January 15, 1998 concerning this subject. Your presenting only the conclusions of the *ad hoc* committee do not afford your reader the benefit of ideas I have brought to this table, which I will not take the time to reiterate here. Nonetheless, my comments and assessment are a part of the record, which I trust will accompany this HIARC report for anyone to see.

P. 12, paragraph 7: Your Panel's Response statement does not adequately embrace the complexities of the comments of the external reviewers. Furthermore, I do not believe it is accurate. I attempted to pull together their conclusions in a paper dated July 27, 1998 submitted to the HIARC Chairman, entitled "Consolidation of External Peer Reviewer's Comments on Malathion non-Cancer Issues, which I trust will be part of the HIARC committee record and fully apparent there. That being the case I will not attempt to suggest revisions to your Panel's Response, but do suggest you revise the statement.

P. 12, paragraph 9: There is nothing *new* offered in citing the *ad hoc* report that serves to compromise the recommendations of the external reviewers, as the *ad hoc* document was submitted to the external reviewers along with my stated objections to the conclusions of the *ad hoc* report, as well as study DERs. In other words, the external reviewers made their recommendations in the face of the *ad hoc* report. I will not take the time here to reiterate my reasons for recommending definitive behavioral effects testing.

P. 13, paragraph 7: The HIARC concluded "..... the entire data base should be examined to see if any peculiarities exist that could serve as a basis for claims of sex-linked sensitivity." I agree with this conclusion and trust there will be follow-up.

P. 14, paragraph 1: In saying that there is no consistent difference in sensitivity of males versus females, you neglected to cite your November 13 *ad hoc* committee report which concluded females were more sensitive. The fundamental question that needs to be address is whether women (girls) are more sensitive than men (boys).

P. 14, paragraph 6: Panel's Report should say in the case of the one member who said no, qualified his no to be applicable as long as the rat study as opposed to the human study serves as the basis for the RfD.

II GENERAL COMMENTS

1) It should be stated somewhere up front in the HIARC report the reason for the external peer review, and exactly what documents were included in the package to that panel of experts, e.g. all malathion DERs, the December 17,

1997 report of the November 6, 1997 HIARC meeting, the November 13, 1997 *ad hoc* subcommittee report, the bulk of the memoranda I submitted to the committee following the November 6, 1997 meeting and *all* of the questions submitted to the panel. I should note all of this information needs to be publicly accessible

2) It is my observation that the external reviewers' conclusions are in many cases complex and are not adequately captured in the brief statements offered as the "Panel's Response" under the various questions in the HIARC draft document of October 27, 1998. I recommend the "Consolidation of External Peer Reviewer's Comments on Malathion non-Cancer Issues" dated July 27, 1998, which was submitted to the committee, as a preferred assessment of the reviewer's comments. This July 27 document must be available as part of the public record.

3) Following the November 6, 1997 meeting on malathion, I have submitted *in good faith* some fourteen or so memoranda to the committee expressing my scientific concerns over the data base. Although the bulk of these were submitted to the external reviewers, it is particularly disappointing that the HIARC has not responded specifically to these, nor do they find any mention in the HIARC report, even though, by in large, they found favor with the external reviewers, suggesting they have scientific merit. These memoranda must be available as part of the public record of the HIARC meetings to consider malathion.

4) When addressing the question of relative sensitivities of young/developing versus adult animals, I noted at the August meeting that two studies on the one-liners showed the young animals to be more sensitive than adults. These studies were: a) a Guideline 81-1 American Cyanamid Company acute oral study on 95% a.i. malathion in the cow, where reportedly the LD50s were 80 mg/kg (calf) and 560 mg/kg (cow); b) an acute intraperitoneal study in male rats on malathion technical (purity not stated, however in reference to the same published work for this study, Substitue Chemical Program 1975 (p. 66) indicates purity as 99%), where the LD50s were 750 mg/kg (adult) versus 340 mg/kg (weanling). There is no acknowledgement of this in the minutes. Also, the Substitute Chenical Program 1975 says: "Young animals appear to be more susceptible to malathion than older animals (Brodeur and DuBoise, 1963)." (p. 67) Along these same lines, I would mention the following publication: Mendoza, C. E. (1976) Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats., Toxicol. Appl. Pharmacol. 35, 229-238. This particular publication has not, to my knowledge, received a formal review. However, it appears in a recognized peer reviewed journal. Among other conclusions reached in this work, the study claims that one-day-old Wistar rats were found to be *nine times* (close, I might add, to that magical 10X factor imposed by Congress) more susceptible to malathion than seventeen-day-old pups. Accordingly, the LD50 for one-day-old rats as performed repeatedly was 209 (ranging 177-250) mg/kg as compared to LD50 values for seventeen-day-old rats of 1806 (ranging 1415-2003) mg/kg. The test material was identified as American Cyanamid 99.3% a.i. malathion. Such information as this serves to support the evidence of enhanced sensitivity of young rats evident in the Guideline reproduction study and in turn support the 10X safety factor imposed under FQPA.

5) An issue not addressed by the HIARC at its August meeting was that of the response of the external reviewers to the question of the adequacy of the malathion data base. This question was posed among a set of preliminary questions to the external reviewers by HED's external peer review coordinator, and I recommended in an August 17, 1998 memorandum to the committee chairman that it be discussed. In essence, the external reviewers identify several data gaps or data deficiencies which are summarized in this August 17 letter. Now whether these deficiencies are data gaps in the strict sense of being unsatisfied end points in Guideline studies (as I believe some are), or inadequacies in the overall assessment of malathion to address health effects concerns, is probably one more of semantics than substance with respect to the intent of Congress to protect infants and children. A most noteable statement along these lines was made by Dr. Dourson, who wrote: *"I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (P. 3 of his June 3, 1998 comments).* **So**

the point I am making here is that it cannot be claimed by HIARC that the no-data-gap qualifier required under FQPA for removal of the 10X safety factor has been met.

6) For the most part, the HIARC has used the same reasoning employed in November 1997 to refute the conclusions/recommendations of the expert panel. There is little evidence the HIARC has been influenced by the external reviewers, whose task it was to weigh in on the differences of opinion between myself and the committee. It is not altogether clear to me why the issues were referred back to the HIARC, but in any case, all of the committee's decisions require review and confirmation outside HED before they become regulatory acceptable. **The following particularly important conclusions are supported by *at least* a consensus of the external reviewers who had the full package of data in hand:**

a) An acute (one-day) end point as high as 0.50 mg/kg is not supported by the data base. It is particularly important this be addressed if the acute (one-day) end point finds use in risk assessments for exposures of up to 7 days;

b) In the absence of assessments of cholinesterase inhibition in young/developing animals versus older animals in developmental and reproduction studies, and the absence of behavioral effects testing in reproduction studies it cannot be interpreted that such studies provide the *reliable* information (as required by Congress) of no increased sensitivity of young animals necessary to discount the 10X safety factor imposed under FQPA for the protection of infants and children. To the extent these studies do not satisfy as *reliable*, the removal of the 10X safety factor imposed under FQPA is not defensible.

c) The actual finding of increased sensitivity of pups versus adults in the reproduction study confirms retention of the 10X safety factor imposed under FQPA for the protection of infants and children (note: I assert an opinion here that a clear consensus among external reviewers would have been expressed in support of this had they been aware that malathion has not been found in milk and that adult animals in the reproduction study were not affected at any dose level, while pup body weight gains were compromized at both the high dose and penultimate dose levels in this study. In further support of a finding that young individuals are more sensitive than older animals to malathion are three LD50 studies cited above showing greater sensitivity of the young. The external reviewers may not have known of these additional studies). Again, in view of the actual findings of enhanced sensitivity of the young, the removal of the 10X safety factor imposed under FQPA would be illegitimate.

d) Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, 50 ppm cannot be concluded to have been a NOEL for the first three months of testing, which is a considerable time frame. In view of this, there is no NOEL for cholinesterase inhibition for females in this study, and hence, in the absence of any additional uncertainty factor, it cannot serve as the basis for the RfD.

e) Cholinesterase methodology may be a problem in this study which needs to be addressed.

f) A shift from the human study to the rat study as the basis for the RfD is unsupported.

g) Use of a mere 10X safety factor to allow for "uncertainties" (knowing of the lack of carboxylesterase in human plasma) in interspecies variability is held to be inadequate should the rat study supplant the human study.

h) The uncertainty factor to be applied to the inhalation end points (intermediate and long term) to compensate

for the absence of a NOEL for nasal and laryngal degeneration/hyperplasia is 10X.

i) A consensus exists among external reviewers that additional assessment of some sort is indicated to address the absence of NOELs in the inhalation study.

j) Retinal tissue histopathology slides should be submitted for independent pathology assessment as called for in the study DER, and retinal tissues slides not taken from lower dose group animals should be submitted, according to Guideline requirements.

k) Additional behavioral effects testing, e.g. developmental neurotoxicity, should be required for malathion as is being done for certain other cholinesterase inhibiting pesticides.

l) Additional testing in animal models should be required to quantitate any gender specific disparity with respect to cholinesterase inhibition.

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